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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1.	Your reference	4-33589P1
		19 JAN 2004
2.	Patent application number *(The Patent Office will fill in this part)*	0401089.8
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND
	Patent ADP number *(if you know it)*	7125487005
	If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND
4.	Title of invention	Organic Compounds
5.	Name of your agent *(If you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Craig McLean Novartis Pharmaceuticals UK Limited Patents and Trademarks Wimblehurst Road Horsham, West Sussex RH12 5AB
	Patents ADP number *(if you know it)*	07181522002

		Country	Priority application number *(if you know it)*	Date of filing (day/month/year)
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *(see note (d))*	No

9.	Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document	
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	Description	16
	Claim(s)	2
	Abstract	
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	Priority documents	
	Translations of priority documents	
	Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
	Request for preliminary examination and search *(Patents Form 9/77)*	1
	Request for substantive examination *(Patents Form 10/77)*	
	Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature — Date

Craig McLean — 19th January 2004

12. Name and daytime telephone number of person to contact in the United Kingdom — Mr. Trevor Drew — (01403) 323069

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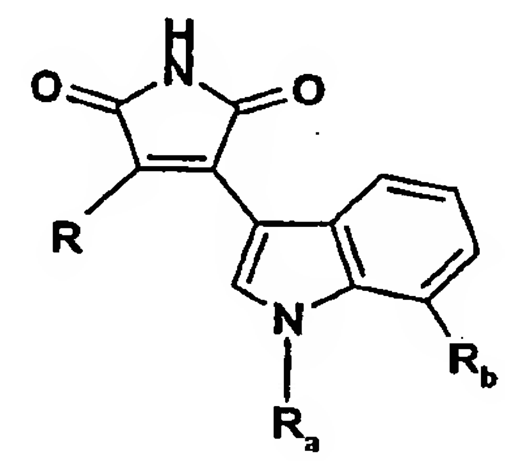
DUPLICATE

Organic Compounds

The present invention relates to indolylmaleimide derivatives, process for their production and pharmaceutical compositions containing them.

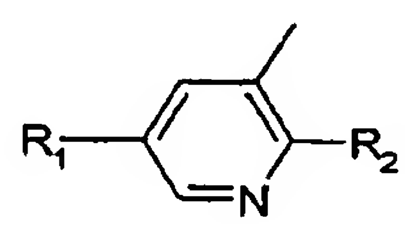
More particularly the present invention provides a compound of formula I



I

wherein

R_a is H; C_{1-4}alkyl; or C_{1-4}alkyl substituted by OH, NH_2, NHC_{1-4}alkyl or N(di-C_{1-4}alkyl)$_2$;
R_b is H; halogen; C_{1-4}alkoxy; or C_{1-4}alkyl, and
R is a radical of formula (a)



(a)

wherein

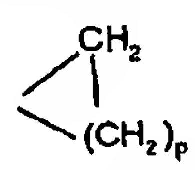
R_1 is a heterocyclic residue; or a radical of formula α

$$-X-R_c-Y \qquad (\alpha)$$

wherein X is a direct bond, O, S or NR_{11} wherein R_{11} is H or C_{1-4}alkyl,
R_c is C_{1-4}alkylene or C_{1-4}alkylene wherein one CH_2 is replaced by CR_xR_y wherein one of R_x and R_y is H and the other is CH_3, each of R_x and R_y is CH_3 or R_x and R_y form together $-CH_2-CH_2-$,
Y is bound to the terminal carbon atom and is selected from OH, $-NR_3R_4$ wherein each of R_3 and R_4, independently, is H, C_{3-6}cycloalkyl, C_{3-6}cycloalkyl-C_{1-4}alkyl, aryl-C_{1-4}alkyl, heteroaryl-C_{1-4}alkyl, C_{2-6}alkenyl or C_{1-4}alkyl optionally substituted on the terminal carbon atom by OH, halogen, C_{1-4}alkoxy or $-NR_5R_6$ wherein each of R_5 and R_6, independently, is H, C_{1-4}alkyl, C_{3-6}cycloalkyl, C_{3-6}cycloalkyl-C_{1-4}alkyl, aryl-C_{1-4}alkyl, or R_3 and R_4 form together with the nitrogen atom to which they are bound a heterocyclic residue; and
R_2 is H; halogen; C_{1-4}alkyl; C_{1-4}alkoxy; CF_3; nitrile; nitro or amino.

Any alkyl or alkyl moiety in e.g. alkoxy may be linear or branched. Halogen may be F, Cl, Br or I, preferably F or Cl. Any aryl may be phenyl or naphthyl, preferably phenyl. Heteroaryl may be a 5 to 8 membered aromatic ring comprising 1 to 4 heteroatoms selected from N, O and S, e.g. pyridyl or pyrimidyl.

By heterocyclic residue as R_1 or Y is meant a three to eight, preferably five to eight, membered saturated, unsaturated or aromatic heterocyclic ring comprising 1 or 2 heteroatoms, preferably selected from N, O and S, and optionally substituted; preferably Y comprises a nitrogen as heteroatom and optionally a second heteroatom, preferably selected from N, O and S, and optionally substituted.

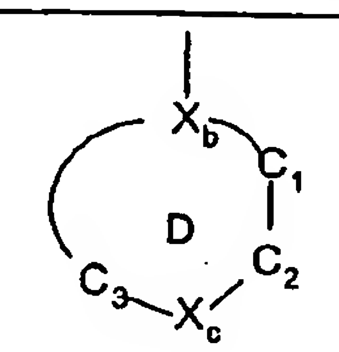
Suitable examples for R_1 or Y include e.g. pyridyl, e.g. 3- or 4-pyridyl, piperidyl, e.g. piperidin-1-yl, 3- or 4-piperidyl, homopiperidyl, piperazinyl, homopiperazinyl, imidazolyl, imidazolidinyl, pyrrolyl, pyrrolidinyl or morpholin-4-yl, optionally substituted, e.g. mono- or polysubstituted. When the heterocyclic residue is substituted, this may be on one or more ring carbon atoms and/or on a ring nitrogen atom when present. Examples of a substituent on a ring carbon atom include e.g. C_{1-4}alkyl e.g. CH_3; C_{3-6}cycloalkyl e.g. cyclopropyl, optionally further substituted by C_{1-4}alkyl;  wherein p is 1,2 or 3, preferably 1; CF_3; halogen; NH_2; $-CH_2-NR_7R_8$ wherein each of R_7 and R_8, independently, is H, C_{1-4}alkyl, or R_7 and R_8 form together with the nitrogen atom to which they are bound a heterocyclic residue or a heteroaryl; $-CH_2-OH$; $-CH_2-O-C_{1-4}$alkyl; $-CH_2$-halogen; or $-CH_2-CH_2$-halogen. Examples of a substituent on a ring nitrogen atom are e.g. C_{1-6}alkyl; acyl, e.g. R'_x-CO wherein R'_x is H, C_{1-6}alkyl or phenyl optionally substituted by C_{1-4}alkyl, C_{1-4}alkoxy or amino, e.g. formyl; C_{3-6}cycloalkyl; C_{3-6}cycloalkyl-C_{1-4}alkyl; phenyl; phenyl-C_{1-4}alkyl e.g. benzyl; a heterocyclic residue, e.g. as disclosed above, e.g. an aromatic heterocyclic residue comprising 1 or 2 nitrogen atoms; or a residue of formula β

$$-R_9-Y' \qquad (\beta)$$

wherein R_9 is C_{1-4}alkylene or C_{2-4}alkylene interrupted by O and Y' is OH, NH_2, $NH(C_{1-4}alkyl)$ or $N(C_{1-4}alkyl)_2$. C_{2-4}alkylene interrupted by O may be e.g. $-CH_2-CH_2-O-CH_2-CH_2-$.

When the substituent on a cyclic nitrogen is a heterocyclic residue, it may be a five or six membered saturated, unsaturated or aromatic heterocyclic ring comprising 1 or 2 heteroatoms, preferably selected from N, O and S. Examples include e.g. 3- or 4-pyridyl, piperidyl, e.g. piperidin-1-yl, 3- or 4-piperidyl, homopiperidyl, piperazinyl, homopiperazinyl, pyrimidinyl, morpholin-4-yl, imidazolyl, imidazolidinyl, pyrrolyl or pyrrolidinyl.

Further examples of heterocyclic residue as R_1 or Y include e.g. a residue of formula (γ)

	(γ)

wherein

the ring D is a 5, 6 or 7 membered saturated, unsaturated or aromatic ring;

X_b is -N=, -C= or –CH-;

X_c is -N=, -NR_f-, -CR_f'= or –CHR_f'- wherein R_f is a substituent as indicated above for a ring nitrogen atom, and R_f' is a substituent as indicated above for a ring carbon atom;

the bond between C_1 and C_2 is either saturated or unsaturated;

each of C_1 and C_2, independently, is a carbon atom which is optionally substituted by one or two substituents selected among those indicated above for a ring carbon atom; and

the line between C_3 and X_b and between C_1 and X_b, respectively, represents the number of carbon atoms as required to obtain a 5, 6 or 7 membered ring D,

whereby when Y is a residue of formula (γ) at least one of X_b and X_c is -N=.

A preferred residue of formula (γ) is one wherein the ring D forms a 1,4-piperazinyl ring optionally C- and/or N-substituted as indicated.

Representative examples of a residue of formula (γ) are e.g. 3- or 4- pyridyl; piperidin-1-yl; 1-N-(C_{1-4}alkyl)- or –(ω-hydroxy-C_{1-4}alkyl)-3-piperidyl; morpholin-4-yl; imidazolyl; pyrrolidinyl; 1-piperazinyl; 2-C_{1-4}alkyl- or –C_{3-6}cycloalkyl-1-piperazinyl ;3-C_{1-4}alkyl- or –C_{3-6}cycloalkyl-1-piperazinyl; 2,2- or 3,5- or 2,5- or 2,6-di(C_{1-4}alkyl)-1-piperazinyl; 3,4,5-tri-(C_{1-4}alkyl)-1-piperazinyl; 4-N-(C_{1-4}alkyl)- or -(ω-hydroxy-C_{1-4}alkyl)- or –(ω–dimethylamino-C_{1-4}alkyl)-1-piperazinyl; 4-N-pyridin-4-yl-1-piperazinyl; 4-N-phenyl- or –C_{3-6}cycloalkyl-1-piperazinyl; 4-N-(C_{1-4}alkyl)- or -(ω-hydroxy-C_{1-4}alkyl)-3-C_{1-4}alkyl- or –3,3–di(C_{1-4}alkyl)-1-piperazinyl; 4-N-(1-C_{1-4}alkyl-C_{3-6}cycloalkyl)-1-piperazinyl; 4-N-formyl-1-piperazinyl; 4-N-pyrimidin-2-yl-1-piperazinyl; or 4-N-C_{1-4}alkyl-1-homopiperazinyl.

When R_a is substituted C_{1-4}alkyl, the substituent is preferably on the terminal carbon atom.

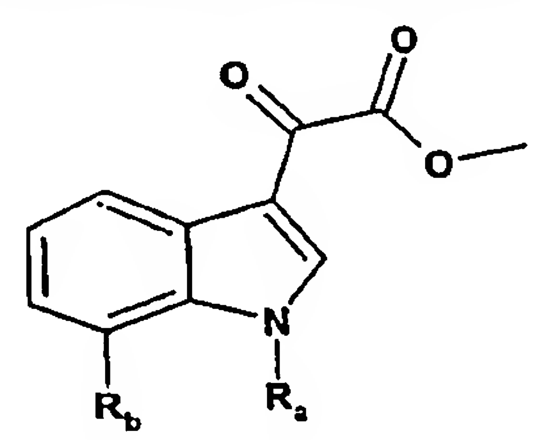
The compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. organic or inorganic acids, for example, hydrochloric acid, acetic acid, trifluoroacetic acid.

It will be appreciated that the compounds of formula I may exist in the form of optical isomers, racemates or diastereoisomers. For example, a ring carbon atom bearing a substituent in the position 3 of the piperazinyl residue is asymmetric and may have the R- or S- configuration. It is to be understood that the present invention embraces all enantiomers and their mixtures. Enantiomers are preferred over racemates. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms as mentioned.

In the compounds of formula I, the following significances are preferred individually or in any sub-combination:

1. R_a is H or methyl;
2. R_b is H, methyl or ethyl;
3. R_1 is a heterocyclic residue, preferably R_1 is piperazin-1-yl optionally substituted by CH_3 in position 3 and/or 4 or in position 3 by ethyl; $-CH_2NR_7R_8$, C_{1-4}alkoxy-C_{1-4}alkyl or halogeno–C_{1-4}alkyl; even more preferably piperazin-1-yl substituted by CH_3 in position 4;
4. R_2 is H; Cl or CF_3.

The present invention also includes a process for the preparation of a compound of formula I which process comprises reacting a compound of formula II



II

wherein R_a and R_b are as defined above,

with a compound of formula III

$$R - CH_2 - CO - NH_2 \qquad (III)$$

wherein R is as defined above,

and, where required, converting the resulting compound of formula I obtained in free form to a salt form or vice versa, as appropriate.

The process may conveniently be effected in the presence of a strong base, e.g. t-BuOK, e.g. as disclosed in WO02/38561 or WO 03/08259, the contents of which being incorporated herein by reference, and as illustrated in the Examples.

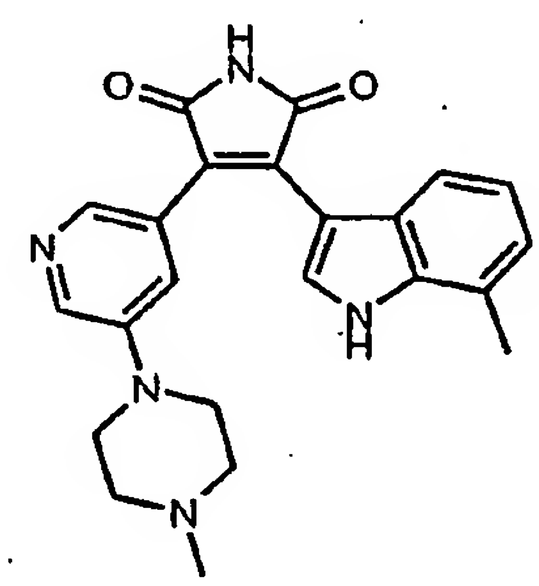
Compounds of formula II and III may be prepared in accordance with known methods, e.g. as disclosed in WO02/38561 or WO 03/08259, the contents of which being incorporated herein by reference, and as illustrated in the Examples.

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known in the art or as described hereafter.

The following examples are illustrative of the invention without any limitation.

RT	=	room temperature
THF	=	tetrahydrofuran
DMF	=	dimethylformamide
EtOAc	=	ethylacetate
$Pd_2(dba)_3$	=	Pd(0)-bis(dibenzylidenacetone)
FCC	=	flash column chromatography
TLC	=	thin layer chromatography

Example 1: 3-(7-Methyl-1H-indol-3-yl)-4-[5-(4-methyl-piperazin-1-yl)-pyridin-3-yl]-pyrrole-2,5-dione



2-[5-(4-Methyl-piperazin-1-yl)-pyridin-3-yl]-acetamide (150 mg, 0.64 mmol) and (7-methyl-1H-indol-3-yl)-oxo-acetic acid methyl ester (209 mg, 0.96 mmol) were dissolved under an atmosphere of argon in a mixture of dry DMF (2 ml) and dry THF (2 ml). A solution of 1.0 M KOtBu in THF (1.9 ml, 1.9 mmol) was then added at RT. After 1 h at 50 °C, TLC analysis indicated complete conversion of starting materials. The reaction mixture was diluted with EtOAc and poured into a saturated aqueous NH_4Cl solution. The organic layer was separated, washed with brine, dried over Na_2SO_4, and the organic solvent was evaporated. The residue was purified by FCC (EtOAc / AcOH / H_2O 600 : 115 : 150) to afford the title compound. ^1H NMR (d_6-DMSO 400 MHz): δ 2.15 (s, 3H), 2.28 – 2.32 (m, 2H), 2.92 – 3.00 (m, 2H), 3.15 (s, 3H), 6.21 (d, J = 9 Hz, 1H), 6.64 (t, J = 9 Hz, 1H), 6.88 (d, J = 9 Hz, 1H), 7.22 (s, 1H), 7.98 (br s, 1H), 8.20 – 8.22 (m, 1H). ES^+-MS: 402.6 $[M + H]^+$.

Preparation of 2-[5-(4-Methyl-piperazin-1-yl)-pyridin-3-yl]-acetamide

[5-(4-Methyl-piperazin-1-yl)-pyridin-3-yl]-acetic acid (1.57 g, 5.78 mmol) and carbonyl diimidazole (1.03 g, 6.36 mmol) were dissolved under an atmosphere of argon in DMF (16 ml). After stirring at RT for 1 h, aqueous NH_4OH (25%, 16 ml) was added, and stirring at RT was continued for 15 minutes. TLC analysis indicated complete consumption of starting material. The aqueous layer was saturated with NaCl and extracted repeatedly with CH_2Cl_2. The organic layers were dried over Na_2SO_4 and concentrated. Purification by FCC (CH_2Cl_2 : MeOH 95 : 5 to 90 : 10 to 80 : 20 to 70 : 30 to 50 : 50 to 25 : 75 to 0 : 100) yielded the title compound. ^{1}H NMR (d_6-DMSO 400 MHz): δ 2.76 (s, 3H), 3.12 – 3.42 (m, 8H), 3.34 (s, 2H), 6.86 – 6.96 (br s, 1H), 7.26 (s, 1H), 7.93 (s, 1H), 8.21 (s, 1H). ES$^+$-MS: 235.4 [M + H]$^+$.

Preparation of [5-(4-Methyl-piperazin-1-yl)-pyridin-3-yl]-acetic acid

[5-(4-Methyl-piperazin-1-yl)-pyridin-3-yl]-acetic acid tert-butyl ester (1.68 g, 5.77 mmol) was dissolved in 4 M HCl in dioxane (28 ml). After 1 h at 60 °C, TLC analysis indicated complete consumption of starting material. The reaction mixture was cooled to RT and diluted with Et_2O. The precipitate was filtered and washed with Et_2O to yield the title compound, which was used in the next reaction without further purification. ^{1}H NMR (d_6-DMSO 400 MHz): δ 2.79 (s, 3H), 3.05 – 4.10 (br m, 8 H), 3.81 (s, 2H), 8.04 (s, 1H), 8.22 (s, 1H), 8.45 (s, 1H). ES$^+$-MS: 236.4 [M + H]$^+$.

Preparation of 2-[5-(4-Methyl-piperazin-1-yl)-pyridin-3-yl]-acetamide [5-(4-Methyl-piperazin-1-yl)-pyridin-3-yl]-acetic acid tert-butyl ester

Potassium phosphate (4.08 g, 19.21 mmol) was dried under high vacuum at 100 °C for 90 minutes. After cooling to RT and venting with argon, $Pd_2(dba)_3$ (70 mg, 0.077 mmol), dicyclohexyl-(2',4',6'-triisopropyl-biphenyl-2-yl)-phosphane (183 mg, 0.38 mmol), a degassed mixture of toluene / tert-butanol (9 : 1, 20 ml) and N-methyl piperazine (1.15 g, 11.53 mmol) were added. The reaction mixture was immersed in a pre-heated oil bath (100 °C). After 2 h at 100 °C, additional $Pd_2(dba)_3$ (70 mg, 0.077 mmol) and dicyclohexyl-(2',4',6'-triisopropyl-biphenyl-2-yl)-phosphane (183 mg, 0.38 mmol) were added. After an additional 2 h at 100°C, TLC analysis indicated complete conversion of starting material. The reaction mixture was cooled to RT, diluted with water and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by FCC (CH_2Cl_2 : MeOH 95 : 5 to 92 : 8 to 88 :12 to 80 : 20) yielded the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.57 (s, 9H),

2.48 (s, 3H), 2.68 – 2.72 (m, 4H), 3.35 – 3.40 (m, 4H), 3.60 (s, 2H), 7.24 – 7.27 (m, 1H), 8.10 (br s, 1H), 8.32 – 8.33 (m, 1H). ES^+-MS: 292.4 $[M + H]^+$.

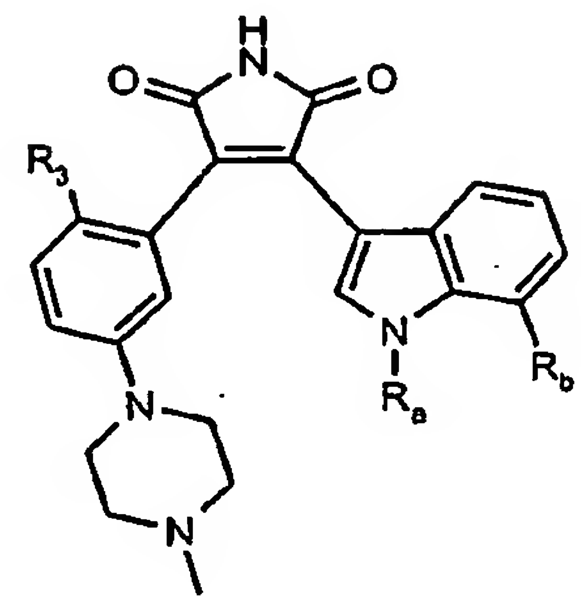
Preparation of (5-Chloro-pyridin-3-yl)-acetic acid tert-butyl ester

$Pd_2(dba)_3$ (928 mg, 1.01 mmol) and (2'-dicyclohexylphosphanyl-biphenyl-2-yl)-dimethyl-amine (838 mg, 2.13 mmol) were added at RT under an atmosphere of argon to a solution of lithium hexamethyl disilazide in toluene (prepared by addition of n-BuLi (1.6 M in hexane, 24.3 ml, 38.85 mmol) to hexamethyl disilazane (6.27 g, 38.85 mmol) in toluene (100 ml) at -78 °C). The mixture was stirred at RT for 10 minutes, then it was cooled to -10 °C. Acetic acid tert-butyl ester (4.12 g, 35.48 mmol) was added during 10 minutes. This mixture was canulated into a cold (- 10 °C) solution of 1,3-dichloropyridine (5.00 g, 33.79 mmol) in toluene (40 ml). The mixture was warmed to RT and stirred for 2 h. TLC analysis indicated substantial conversion of starting material. Water was added, the resulting slurry was filtered, and the filtrate was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by FCC (hexane / EtOAc 100 : 0 to 92 : 8 to 85 : 15) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.39 (s, 9H), 3.47 (s, 2H), 7.58 – 7.60 (m, 1H), 8.31 (s, 1H), 8.41 – 8.42 (m, 1H). ES^+-MS: 228.3 $[M + H]^+$.

By following the procedure of Example 1, but using the appropriate starting materials, the compounds of formula A wherein R_a, R_b, and R_3 are as indicated in Table 1 below, may be obtained.

Table 1

	R_3	R_a	R_b	MS
2.	Cl	H	CH_3	MH^+ 436
3.	H	CH_3	H	MH^+ 402
4.	H	H	H	MH^+ 388
5.	CF_3	H	CH_3	MH^+ 470
6.	CF_3	H	H	MH^+ 456
7.	CF_3	CH_3	H	MH^+ 470



A

The compounds of formula I in free form or in pharmaceutically acceptable salt form exhibit valuable pharmacological properties, e.g. inhibiting Protein Kinase C (PKC), e.g. PKC isoforms like α, β, δ, ε, η or θ activity, inhibiting T-cell activation and proliferation, e.g. by inhibiting production by T-cells or cytokines, e.g. IL-2, by inhibiting the proliferative response of T-cells to cytokines, e.g. IL-2, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. In vitro

1. Protein Kinase C assay

The compounds of formula I are tested for their activity on different PKC isoforms according to a published method (D. Geiges et al. Biochem. Pharmacol. 1997;53:865-875) The assay is performed in a 96-well polypropylene microtiterplate (Costar 3794) that has been previously siliconized with Sigmacote (Sigma SL-2). The reaction mixture (50 µl) contains 10 µl of the relevant PKC isozyme together with 25 µl of the test compound and 15 µl of a mix solution that contains 200 µg/ml protamine sulfate, 10 mM $Mg(NO_3)_2$, 10 µM ATP (Boehringer 519987) and 3750 Bq of ^{33}P-ATP (Hartmann Analytic SFC301, 110TBq/mmol) in 20 mM Tris-buffer pH 7.4 + 0.1% BSA. Incubation is performed for 15 min at 32°C in a microtiterplate shaking incubator (Biolabo Scientific Instruments). Reaction is stopped by adding 10 µl of 0.5 M Na_2EDTA, pH 7.4. 50 µl of mixture are pipetted onto a pre-wetted phosphocellulose paper (Whatmann 3698-915) under gentle pressure. Non-incorporated ATP is washed away with 100 µl bi-dist H_2O. The paper is washed twice in 0.5% H_3PO_4 for 15 min followed by 5 min in EtOH. Thereafter the paper is dryed and placed in an omnifilter (Packard 6005219), and overlayed with 10 µl/well of Microscint-O (Packard 6013611) before counting in a Topcount radioactivity counter (Packard). IC_{50} measurement is performed on a routine basis by incubating a serial dilution of inhibitor at concentrations ranging between 1-

1000 µM according to the method described above. IC_{50} value are calculated from the graph by sigmoidal curve fitting.

2. Protein Kinase C θ Assay

Human recombinant PKCθ is used under the assay conditions as described above. In this assay, compounds of formula I inhibit PKC θ with an $IC_{50} \leq 1$ µM.

3. Protein Kinase Cα Assay

Human recombinant PKCα was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCα with an $IC_{50} \leq 1$ µM.

4. Protein Kinase Cβ1 Assay

Human recombinant PKCβ1 was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCβ1 with an $IC_{50} \leq 1$ µM.

5. Protein Kinase Cδ Assay

Human recombinant PKCδ was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCδ with an $IC_{50} \leq 1$ µM.

6. Protein Kinase Cε Assay

Human recombinant PKCε was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCε with an $IC_{50} \leq 1$ µM.

7. Protein Kinase Cη Assay

Human recombinant PKCη was obtained from PanVera and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCη with an $IC_{50} \leq 1$ µM.

8. CD28 costimulation assay

The assay is performed with Jurkat cells transfected with a human interleukin-2 promoter/reporter gene construct as described by Baumann G et al. in Transplant. Proc. 1992;24:43-8, the ß-galactosidase reporter gene being replaced by the luciferase gene (de Wet J., et al., Mol. Cell Biol. 1987, 7(2), 725-737). Cells are stimulated by solid phase-coupled antibodies or phorbol myristate acetate (PMA) and the Ca^{++} ionophore ionomycin as

follows. For antibody-mediated stimulation Microlite TM1 microtiter plates (Dynatech) are coated with 3 μg/ml goat anti-mouse IgG Fc antibodies (Jackson) in 55 μl phosphate-buffered saline (PBS) per well for three hours at RT. Plates are blocked after removing the antibodies by incubation with 2% bovine serum albumin (BSA) in PBS (300 μl per well) for 2 hours at RT. After washing three times with 300 μl PBS per well, 10 ng/ml anti-T cell receptor antibodies (WT31, Becton & Dickinson) and 300 ng/ml anti-CD28 antibodies (15E8) in 50 μl 2% BSA/PBS are added as stimulating antibodies and incubated overnight at 4°C. Finally the plates are washed three times with 300 μl PBS per well. Seven three-fold serial dilutions of test compounds in duplicates in assay medium (RPMI 1640/10% fetal calf serum (FCS) containing 50 μM 2-mercaptoethanol, 100 units/ml penicillin and 100 μg/ml streptomycin) are prepared in separate plates, mixed with transfected Jurkat cells (clone K22 290_H23) and incubated for 30 minutes at 37°C in 5% CO_2. 100 μl of this mixture containing 1×10^5 cells are then transferred to the antibody-coated assay plates. In parallel 100 μl are incubated with 40 ng/ml PMA and 2 μM ionomycin. After incubation for 5.5 hours at 37°C in 5% CO_2, the level of luciferase is determined by bioluminescence measurement. The plates are centrifuged for 10 min at 500 g and the supernatant is removed by flicking. Lysis buffer containing 25 mM Tris-phosphate, pH 7.8, 2 mM DTT, 2 mM 1.2-diaminocyclohexane-N,N,N',N-tetraacetic acid, 10 % (v/v) glycerol and 1 % (v/v) Triton X-100 is added (20 μl per well). The plates are incubated at RT for 10 minutes under constant shaking. Luciferase activity is assessed with a bioluminescence reader (Labsystem, Helsinki, Finland) after automatic addition of 50 μl per well luciferase reaction buffer containing 20 mM Tricine, 1.07 mM $(MgCO_3)_4Mg(OH)_2 x 5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM DTT, 270 μM coenzyme A, 470 μM luciferin (Chemie Brunschwig AG), 530 μM ATP, pH 7.8. Lag time is 0.5 seconds, total measuring time is 1 or 2 seconds. Low control values are light units from anti-T cell receptor- or PMA-stimulated cells, high controls are from anti-T cell receptor/anti-CD28- or PMA/ionomycin-stimulated cells without any test sample. Low controls are subtracted from all values. The inhibition obtained in the presence of a test compound is calculated as percent inhibition of the high control. The concentration of test compounds resulting in 50% inhibition (IC_{50}) is determined from the dose-response curves. In this assay, compounds of formula I inhibit anti-T cell receptor/anti-CD28 and PMA/ionomycin stimulated Jurkat cells with an $IC_{50} \leq 1$ μM.

9. <u>Allogeneic Mixed Lymphocyte Reaction (MLR)</u>

The two-way MLR is performed according to standard procedures (J. Immunol. Methods, 1973, 2, 279 and Meo T. et al., Immunological Methods, New York, Academic Press, 1979, 227-39). Briefly, spleen cells from CBA and BALB/c mice (1.6×10^5 cells from each strain per well in flat bottom tissue culture microtiter plates, 3.2×10^6 in total) are incubated in RPMI medium containing 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco BRL, Basel, Switzerland), 50 µM 2-mercaptoethanol (Fluka, Buchs, Switzerland) and serially diluted compounds. Seven three-fold dilution steps in duplicates per test compound are performed. After four days of incubation 1 µCi ^{3}H-thymidine is added. Cells are harvested after an additional five-hour incubation period, and incorporated ^{3}H-thymidine is determined according to standard procedures. Background values (low control) of the MLR are the proliferation of BALB/c cells alone. Low controls are subtracted from all values. High controls without any sample are taken as 100% proliferation. Percent inhibition by the samples is calculated, and the concentrations required for 50% inhibition (IC_{50} values) are determined.

10. <u>Inhibition of GSK-3β</u>

The GSK-3β bibding assay is performed in 50µl reactions in 96 well polypropylene plate, each reaction containing 20mM magnesium chloride, 40 µM ATP, 2mM DTT, 88.5µM biotinylated and phosphorylated CREB-peptide substrate (biotin-KRREILSRRPS(PO_4)YR-OH ; Q. M. Wang et al., J. Biol. Chem. 269, 14566-14574, 1994), [γ-^{33}P]ATP (1µCi) and 2µl of the compound to be tested in DMSO(various concentrations). 15µl of GSK-3β (various concentrations) is added and the mixture is incubated at 30°C for 1 hour. The reaction is stopped by transferring 25 µl of the mixture to a phosphocellulose plate containing 130µl of 1.85% phosphoric acid. The free radionucleotides in the membrane are washed off under vacuum with 1.85% phosphoric acid (5 times). After the last wash, the plate is transferred to an adaptor plate and 50µl of scintillation cocktail (Microscint-20, Packard, cat. # 20-133) is added to each well and the amount of radioactivity is counted in a top counter. Compounds of formula I are active in this assay.

B. In vivo

<u>Rat Heart transplantation</u>

The strain combination used: Male Lewis (RT^1 haplotype) and BN (RT^1 haplotype). The animals are anaesthetised using inhalational isofluorane. Following heparinisation of the donor rat through the abdominal inferior vena cava with simultaneous exsanguination via the aorta, the chest is opened and the heart rapidly cooled. The aorta is ligated and divided distal to the first branch and the brachiocephalic trunk is divided at the first bifurcation. The

left pulmonary artery is ligated and divided and the right side divided but left open. All other vessels are dissected free, ligated and divided and the donor heart is removed into iced saline.

The recipient is prepared by dissection and cross-clamping of the infra-renal abdominal aorta and vena cava. The graft is implanted with end-to-side anastomoses, using 10/0 monofilament suture, between the donor brachiocephalic trunk and the recipient aorta and the donor right pulmonary artery to the recipient vena cava. The clamps are removed, the graft tethered retroabdominally, the abdominal contents washed with warm saline and the animal is closed and allowed to recover under a heating lamp. Graft survival is monitored by daily palpation of the beating donor heart through the abdominal wall. Rejection is considered to be complete when heart beat stops. Increases of graft survival are obtained in animals treated with a compound of formula I administered orally at a daily dose of 1 to 30 mg/kg bid.

Graft v. Host Model

Spleen cells ($2x10^7$) from Wistar/F rats are injected subcutaneously into the right hind footpad of (Wistar/F x Fischer 344)F_1 hybrid rats. The left footpad is left untreated. The animals are treated with the test compounds on 4 consecutive days (0-3). The popliteal lymph nodes are removed on day 7, and the weight differences between two corresponding lymph nodes are determined. The results are expressed as the inhibition of lymph node enlargement (given in percent) comparing the lymph node weight differences in the experimental groups to the weight difference between the corresponding lymph nodes from a group of animals left untreated with a test compound.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by T lymphocytes and/or PKC, e.g. acute or chronic rejection of organ or tissue allo- or xenografts, graft versus host diseases, atherosclerosis, vascular occlusion due to vascular injury such as angioplasty, restenosis, obesity, syndrome X, impaired glucose tolerance, polycystic ovary syndrome, hypertension, heart failure, chronic obstructive pulmonary disease, CNS diseases such as Alzheimer disease or amyotrophic lateral sclerosis, cancer, infectious diseases such as AIDS, septic shock or adult respiratory distress syndrome, ischemia/reperfusion injury e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, or traumatic shock, e.g. traumatic brain injury. The compounds of formula I are also useful in the treatment and/or prevention of T-cell mediated acute or chronic inflammatory diseases or disorders or autoimmune diseases e.g.

rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, Hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes type I or II and the disorders associated therewith, respiratory diseases such as asthma or inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, cutaneous manifestations of immunologically-mediated disorders or illnesses, inflammatory and hyperproliferative skin diseases (such as psoriasis, atopic dermatitis, allergic contact dermatitis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis), inflammatory eye diseases, e.g. Sjoegren's syndrome, keratoconjunctivitis or uveitis, inflammatory bowel disease, Crohn's disease or ulcerative colitis. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.1 to about 100 mg/kg body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 2000 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form.

The compounds of formula I may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent. Unit dosage forms for oral administration contain, for example, from about 0.1 mg to about 500 mg of active substance.

Topical administration is e.g. to the skin. A further form of topical administration is to the eye.

The compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by T lymphocytes and/or PKC or GSK-3β, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

2. A compound of formula I, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 and 1.2 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 and 1.2 above comprising a compound of formula I in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula I or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 and 1.2 above.

Compounds of formula I may be administered as the sole active ingredient or together with other drugs in immunomodulating regimens or other anti-inflammatory agents e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders. For example, they may be used in combination with cyclosporines, or ascomycines or their immunosuppressive analogs or derivatives, e.g. cyclosporin A, ISA Tx247, FK-506, ABT-281, ASM 981; an mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578, or a rapalog, e.g. AP23573, AP23464, AP23675, AP23841, TAFA-93, biolimus 7 or biolimus 9 etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; an EDG receptor agonist having accelerating lymphocyte homing properties, e.g. FTY 720 or an analogue thereof; leflunomide or analogs thereof; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or analogs thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD 11a/CD18, CD7, CD25, CD 27, B7, CD40, CD45, CD58, CD 137, ICOS, CD150 (SLAM), OX40, 4-1BB or their ligands, e.g. CD154; or other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant

thereof, e.g. LEA29Y, or other adhesion molecule inhibitors, e.g. mAbs or low molecular weight inhibitors including LFA-1 antagonists, Selectin antagonists and VLA-4 antagonists. Compounds of formula I may also be administered together with an antiproliferative drug, e.g. a chemotherapeutic drug, e.g. as used in cancer treatment, including but not limited to aromatase inhibitors, antiestrogens, topoisomerase I inhibitors, topoisomerase II inhibitors, microtubule active agents, alkylating agents, histone deacetylase inhibitors, farnesyl transferase inhibitors, COX-2 inhibitors, MMP inhibitors, mTOR inhibitors, antineoplastic antimetabolites, platin compounds, compounds decreasing the protein kinase activity and further anti-angiogenic compounds, gonadorelin agonists, anti-androgens, bengamides, bisphosphonates, antiproliferative antibodies and temozolomide, or with an anti-diabetic drug, an insulin secretagogue or insulin secretion enhancer, e.g. a sulphonyl urea, e.g. tolbutamide, chlorpropamide, tolazamide, acetohexamide, 4-chloro-N-[(1-pyrolidinyl-amino)carbonyl]-benzensulfonamide (glycopyramide), glibenclamide (glyburide), gliclazide, 1-butyl-3-metanilylurea, carbutamide, glibonuride, glipizide, gliquidone, glisoxepid, glybuthiazole, glibuzole, glyhexamide, glymidine, glypinamide, phenbutamide or tolylcyclamide, an oral insulinotropic agent derivative, e.g. a short acting insulin enhancer, e.g. meglitinide, repaglinide, a phenyl acetic acid derivative, e.g.nateglinide, a DPP IV inhibitor, e.g. 1-{2-[(5-cyanopyridin-2-yl)amino]ethylamino}acetyl-(2S)-cyano-pyrrolidine di-hydrochloride, LAF237, GLP-1 or a GLP-1 agonist analog, or an insulin sensitizer e.g. a peroxisome proliferator activated receptor γ agonist (PPARγ), e.g. a glitazone, a non-glitazone type such as a N-(2-benzoylphenyl)-L-tyrosine analogue, e.g. GI-262570, or an oxolidinedione, e.g. JTT501, a dual PPARγ/PPARα agonist, e.g. DRF-554158, NC-2100 or NN-622, a retinoid X receptor agonist or a rexinoid, e.g. 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid, 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-2-carbonyl]-benzoic acid, 9-cis retinoic acid or an analog, derivative or a pharmaceutically acceptable salt thereof, in diabetes therapy.

In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I in free form or in pharmaceutically acceptable salt form, and a second drug substance, said second drug substance being an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic drug, e.g. as indicated above.

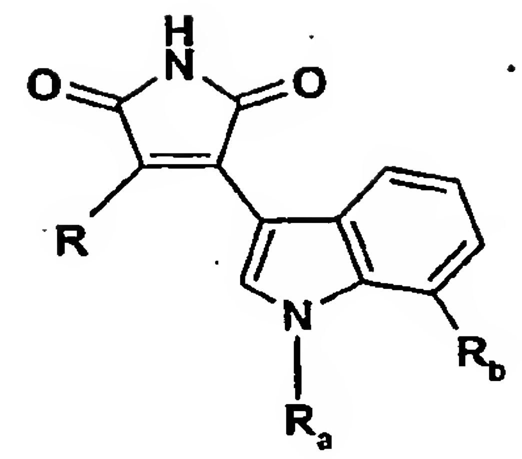
6. A therapeutic combination, e.g. a kit, comprising a) an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I in free form or in pharmaceutically acceptable salt form, and b) at least one second agent selected from an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative and anti-diabetic drug. Component a) and component b) may be used concomitantly or in sequence. The kit may comprise instructions for its administration.

Where an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I, is administered in conjunction with other immunosuppressive/immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic therapy, e.g. for preventing or treating acute or chronic graft rejection or inflammatory or autoimmune disorders as hereinabove specified, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a cyclosporine, on the specific drug employed, on the condition being treated and so forth.

Compounds of formula I have an interesting pharmacokinetic profile and interesting in vitro and in vivo activities.

CLAIMS

1. A compound of formula I



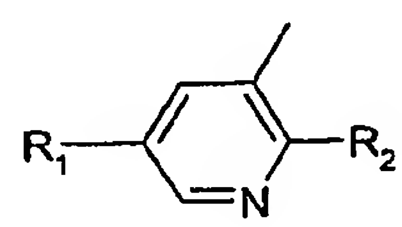
I

wherein

R_a is H; C_{1-4}alkyl; or C_{1-4}alkyl substituted by OH, NH_2, NHC_{1-4}alkyl or N(di-C_{1-4}alkyl)$_2$;

R_b is H; halogen; C_{1-6}alkoxy; or C_{1-6}alkyl, and

R is a radical of formula (a)



(a)

wherein

R_1 is a heterocyclic residue; or a radical of formula α

$$-X-R_c-Y \qquad (\alpha)$$

wherein X is a direct bond, O, S or NR_{11} wherein R_{11} is H or C_{1-4}alkyl,

R_c is C_{1-4}alkylene or C_{1-4}alkylene wherein one CH_2 is replaced by CR_xR_y wherein one of R_x and R_y is H and the other is CH_3, each of R_x and R_y is CH_3 or R_x and R_y form together $-CH_2-CH_2-$,

Y is bound to the terminal carbon atom and is selected from OH, $-NR_3R_4$ wherein each of R_3 and R_4, independently, is H, C_{3-6}cycloalkyl, C_{3-6}cycloalkyl-C_{1-4}alkyl, aryl-C_{1-4}alkyl, heteroaryl-C_{1-4}alkyl, C_{2-6}alkenyl or C_{1-4}alkyl optionally substituted on the terminal carbon atom by OH, halogen, C_{1-4}alkoxy or $-NR_5R_6$ wherein each of R_5 and R_6, independently, is H, C_{1-4}alkyl, C_{3-6}cycloalkyl, C_{3-6}cycloalkyl-C_{1-4}alkyl, aryl-C_{1-4}alkyl, or R_3 and R_4 form together with the nitrogen atom to which they are bound a heterocyclic residue; and

R_2 is H; halogen; C_{1-4}alkyl; C_{1-4}alkoxy; CF_3; nitrile; nitro or amino;

or a salt thereof,

a process for its preparation, its use as a pharmaceutical, a pharmaceutical composition containing such a compound or a pharmaceutically acceptable salt thereof, a method of treatment or prevention using such a compound or a

pharmaceutically acceptable salt thereof, or a pharmaceutical combination comprising such a compound or a pharmaceutically acceptable salt thereof, substantially as herein defined and/or described.

2. A compound substantially as described in the Examples.

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19 JAN 2004

2. Patent application number *(The Patent Office will fill in this part)* — 0401090.6

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

NOVARTIS AG
LICHTSTRASSE 35
4056 BASEL
SWITZERLAND

Patent ADP number *(if you know it)* — 7254870005

If the applicant is a corporate body, give the country/state of its incorporation — SWITZERLAND

4. Title of invention — Organic Compounds

5. Name of your agent *(If you have one)* — Craig McLean

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Novartis Pharmaceuticals UK Limited
Patents and Trademarks
Wimblehurst Road
Horsham, West Sussex
RH12 5AB

Patents ADP number *(if you know it)* — 07181522002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing (day/month/year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (day/month/year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* — No

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body.

(see note (d))

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Description	24
Claim(s)	1
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Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature	Date
Craig McLean	19th January 2004

12. Name and daytime telephone number of person to contact in the United Kingdom — Mr. Trevor Drew (01403) 323069

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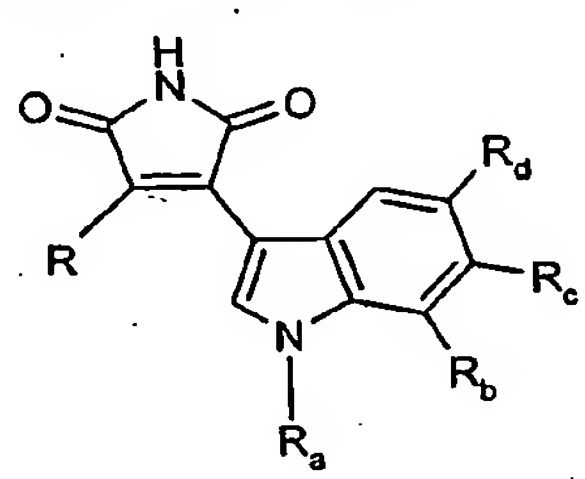
DUPLICATE

Organic Compounds

The present invention relates to indolylmaleimide derivatives, process for their production and pharmaceutical compositions containing them.

More particularly the present invention provides a compound of formula I



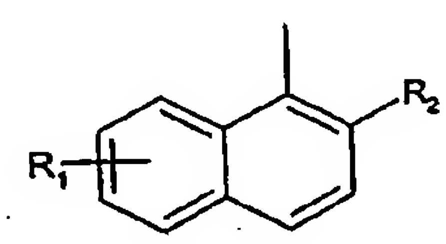
I

wherein

R_a is H; C_{1-4}alkyl; or C_{1-4}alkyl substituted by OH, NH_2, NHC_{1-4}alkyl or N(di-C_{1-4}alkyl)$_2$;

one of R_b, R_c and R_d is halogen; C_{1-4}alkoxy; or C_{1-4}alkyl; and the other two substituents are H; or R_b, R_c and R_d are all H; and

R is a radical of formula (a)



(a)

wherein

R_1 is $-(CH_2)_n-NR_3R_4$ and R_1 is in position 6 or 7,

wherein

each of R_3 and R_4, independently, is H or C_{1-4}alkyl; or R_3 and R_4 form together with the nitrogen atom to which they are bound a heterocyclic residue;

n is 0, 1 or 2; and

R_2 is H; halogen; C_{1-4}alkyl; CF_3; OH; SH; NH_2; C_{1-4}alkoxy; C_{1-4}alkylthio; NHC_{1-4}alkyl; N(di-C_{1-4}alkyl)$_2$ or CN.

The compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. organic or inorganic acids, for example, hydrochloric acid, acetic acid, trifluoroacetic acid.

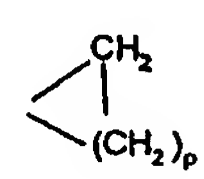
It will be appreciated that the compounds of formula I may exist in the form of optical isomers, racemates or diastereoisomers. For example, a ring carbon atom bearing a substituent in the position 3 of the piperazinyl residue is asymmetric and may have the R- or

S- configuration. It is to be understood that the present invention embraces all enantiomers and their mixtures. Enantiomers are preferred over racemates. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms as mentioned.

Alkyl or alkoxy may be straight or branched.

Halogen may be F, Cl, Br or I, preferably F, Cl or Br.

By heterocyclic residue is meant a three to eight, preferably five to eight, membered saturated, unsaturated or aromatic heterocyclic ring comprising 1 or 2 heteroatoms, preferably selected from N, O and S, and optionally substituted. Suitable examples include e.g. pyridyl, e.g. 3- or 4-pyridyl, piperidyl, e.g. piperidin-1-yl, 3- or 4-piperidyl, homopiperidyl, piperazinyl, homopiperazinyl, morpholin-4-yl, imidazolyl, imidazolidinyl, pyrrolyl or pyrrolidinyl, optionally substituted, e.g. mono- or polysubstituted. When the heterocyclic residue is substituted, this may be on one or more ring carbon atoms and/or on a ring nitrogen atom when present. Examples of a substituent on a ring carbon atom include e.g. C_{1-4}alkyl e.g. CH_3;

C_{3-6}cycloalkyl e.g. cyclopropyl, optionally further substituted by C_{1-4}alkyl;  wherein p is 1,2 or 3, preferably 1; CF_3; halogen; OH; NH_2; $-CH_2-NH_2$; $-CH_2-OH$; piperidin-1-yl; or pyrrolidinyl. Examples of a substituent on a ring nitrogen atom are e.g. C_{1-6}alkyl; acyl, e.g. R'_x-CO wherein R'_x is H, C_{1-6}alkyl or phenyl optionally substituted by C_{1-4}alkyl, C_{1-4}alkoxy or amino, e.g formyl; C_{3-6}cycloalkyl; C_{3-6}cycloalkyl-C_{1-4}alkyl; phenyl; phenyl-C_{1-4}alkyl e.g. benzyl; a heterocyclic residue, e.g. as disclosed above, e.g. an aromatic heterocyclic residue comprising 1 or 2 nitrogen atoms; or a residue of formula β

$$-R_5-Y' \qquad (\beta)$$

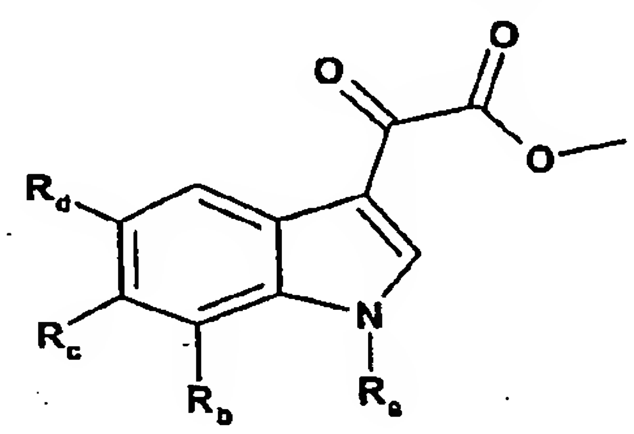
wherein R_5 is C_{1-4}alkylene or C_{2-4}alkylene interrupted by O and Y' is OH, NH_2, $NH(C_{1-4}alkyl)$ or $N(C_{1-4}alkyl)_2$.

In the compounds of formula I, the following significances are preferred individually or in any sub-combination:

1. R_a is H or methyl;
2. one of R_b, R_c and R_d is methyl or ethyl and the other two substituents are H; or R_b, R_c and R_d are all H;
3. R_2 is H, Cl, or methyl;
4. n is 1; and

5. each of R_3 and R_4, independently, is H, methyl, ethyl or *i*-propyl; or R_3 and R_4 form together with the nitrogen atom to which they are bound a heterocyclic residue e.g. an optionally substituted piperazinyl or pyrrolidinyl.

The present invention also includes a process for the preparation of a compound of formula I which process comprises reacting a compound of formula II



II

wherein R_a to R_d are as defined above,

with a compound of formula III

$$R - CH_2 - CO - NH_2 \qquad \text{(III)}$$

wherein R is as defined above,

and, where required, converting the resulting compound of formula I obtained in free form to a salt form or vice versa, as appropriate.

The process may conveniently be effected in the presence of a strong base, e.g. t-BuOK, e.g. as disclosed in WO02/38561, the contents of which being incorporated herein by reference, and as illustrated in the Examples.

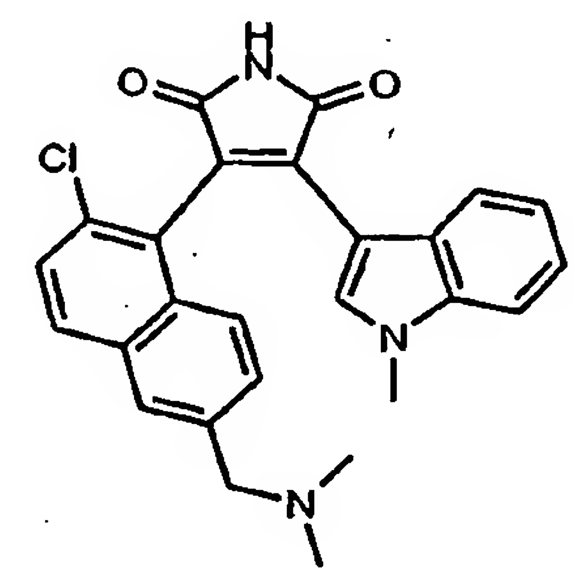
Compounds of formula II and III may be prepared in accordance with known methods, e.g. as disclosed in WO02/38561 or WO 03/08259, the contents of which being incorporated herein by reference, and as illustrated in the Examples.

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known in the art or as described hereafter.

The following examples are illustrative of the invention without any limitation.

RT	=	room temperature
THF	=	tetrahydrofuran
DMF	=	dimethylformamide
EtOAc	=	ethylacetate
FCC	=	flash column chromatography
TLC	=	thin layer chromatography

Example 1: 3-(2-Chloro-6-dimethylaminomethyl-naphthalen-1-yl)-4-(1-methyl-1H-indol-3-yl)-pyrrole-2,5-dione



Activated 3Å molecular sieve (50 mg) was added to a solution of 2-(2-Chloro-6-dimethylaminomethyl-naphthalen-1-yl)-acetamide (54.6 mmol, 0.20 mmol) and (1-Methyl-1H-indol-3-yl)-oxo-acetic acid methyl ester (55.7 mg, 0.26 mmol) in dry THF (2.5 ml) under an atmosphere of argon. A solution of 1.0 M KOtBu in THF (0.59 ml, 0.59 mmol) was then added in one portion at RT. After 30 minutes at RT, TLC analysis indicates complete conversion of starting materials. The reaction mixture was diluted with EtOAc and poured into a saturated aqueous NH_4Cl solution. The organic layer was separated, washed with brine, dried over Na_2SO_4, and the organic solvent was evaporated. The residue was purified by FCC (EtOAc / AcOH / H_2O 700 : 110 : 90) to afford the title compound. ^1H NMR (d_6-DMSO, 400 MHz): δ 2.12 (s, 6H), 3.46 (s, 2H), 3.82 (s, 3H), 6.16 (d, J = 8.8 Hz, 1H), 6.45 – 6.51 (m, 1H), 6.96 – 7.02 (m, 1H), 7.32 – 7.40 (m, 2H), 7.60 – 7.68 (m, 2H), 7.88 (s, 1H), 8.06 (d, J = 10 Hz, 1H), 8.15 (s, 1H). ES^+-MS: 445.5, 446.6 $[M + H]^+$.

Preparation of 2-(2-Chloro-6-dimethylaminomethyl-naphthalen-1-yl)-acetamide

(2-Chloro-6-dimethylaminomethyl-naphthalen-1-yl)-acetic acid (276 mg, 0.99 mmol) was dissolved under an atmosphere of argon in DMF (3 ml). 1,1-Carbonyl diimidazole (177 mg, 1.09 mmol) was added, and the clear solution was stirred at RT for 3 h. A conc. aqueous solution of ammonia (25%, 6 ml) was added, and stirring was continued for 10 minutes at RT. TLC analysis indicates complete consumption of starting material. The reaction mixture was poured on water. The aqueous layer was extracted with EtOAc, which was then washed with brine and dried over Na_2SO_4. After removal of solvent, the residue was found to be pure title compound, with no need of purification. ^1H NMR (d_6-DMSO, 400 MHz): δ 2.18 (s, 6H), 3.53 (s, 2H), 4.08 (s, 2H), 6.96 – 7.08 (br, 2H), 7.48 – 7.68 (m, 2H), 7.78 – 7.86 (m, 2H), 7.96 – 8.00 (d, J = 10 Hz, 1H). ES^+-MS: 277.3, 279.2 $[M + H]^+$.

Preparation of (2-Chloro-6-dimethylaminomethyl-naphthalen-1-yl)-acetic acid

(2-Chloro-6-dimethylaminomethyl-naphthalen-1-yl)-acetic acid ethyl ester (223 mg, 0.73 mmol) was dissolved in dioxane (2.6 ml). Water (0.96 ml) and lithium hydroxide (21 mg, 0.88 mmol) were then added, and the reaction mixture was warmed to 60 °C for 4 h. HPLC analysis indicates complete conversion of starting material. The reaction was diluted with water, adjusted to pH 6 – 7 by addition of 1 M aqueous $NaHSO_4$, and extracted with EtOAc. The water layer was then concentrated, and the solid residue was repeatedly extracted with MeOH to yield pure title compound. ES^+-MS: 278.3, 280.1 $[M + H]^+$.

Preparation of (2-Chloro-6-dimethylaminomethyl-naphthalen-1-yl)-acetic acid ethyl ester

Dimethylamine (5.6 M solution in EtOH, 0.28 ml, 1.53 mmol) was added under an atmosphere of argon to a solution of (2-chloro-6-formyl-naphthalen-1-yl)-acetic acid ethyl ester (284 mg, 1.02 mmol) in THF (10 ml). The mixture was stirred at RT for 18 h, before a solution of sodium cyanoborohydride (78 mg, 1.23 mmol) in MeOH (2 ml) and glacial acetic acid (0.29 ml, 5.13 mmol) were added. After stirring at RT for 1 h, TLC analysis indicates complete consumption of starting material. The reaction mixture was diluted with water and adjusted to pH 8 – 9 by the addition of conc. aq. $NaHCO_3$ solution. Extraction with EtOAc, washing with brine, drying over Na_2SO_4 and removal of solvent yielded the crude reaction product. Purification by FCC (CH_2Cl_2 / EtOH / NH_3 190:9:1) afforded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, J = 9 Hz, 3H), 2.30 (s, 6H), 3.59 (s, 2H), 4.18 (q, J = 9 Hz, 2H), 4.30 (s, 2H), 7.49 (d, J = 10 Hz, 1H), 7.54 – 7.58 (m, 1H), 7.69 – 7.76 (m, 2H), 7.91 (d, J = 10 Hz, 1H). ES^+-MS: 306.4, 308.3 $[M + H]^+$.

Preparation of (2-Chloro-6-formyl-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-6-cyano-naphthalen-1-yl)-acetic acid ethyl ester (1.39 g, 5.07 mmol) was dissolved in a mixture of water (17 ml), pyridine (33 ml) and glacial acetic acid (17 ml). Sodium hypophosphite (4.30 g, 40.62 mmol) and Raney nickel (3.2 g) were then added at RT. The reaction mixture was heated to 100 °C for 1 h. TLC analysis indicates complete consumption of starting material. The reaction mixture was cooled to RT and filtered through Celite. After addition of silica gel, the solvents were removed on a rotary evaporator. Purification by FCC (hexane / EtOAc 5 : 1) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.17 (t, J = 8 Hz, 3H), 4.10 (q, J = 8 Hz, 2H), 4.24 (s, 2H), 7.52 (d, J = 10 Hz, 1H), 7.82 (d, J = 10 Hz, 1H), 7.94 – 7.98 (m, 2H); 8.26 (s, 1H), 10.09 (s, 1H). ES^--MS: 275.2, 277.3 $[M - H]^-$.

Preparation of (2-Chloro-6-cyano-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-6-trifluoromethanesulfonyloxy-naphthalen-1-yl)-acetic acid ethyl ester (3.59 g, 9.04 mmol) was dissolved in DMF (30 ml) under an atmosphere of argon. After addition of palladium(0) tetrakis(triphenylphosphane) (418 mg, 0.36 mmol) and zinc(II) cyanide (2.12 g, 18.09 mmol), the reaction mixture was heated to 125 °C. After 1 h, TLC analysis indicates complete consumption of starting material. The suspension was cooled to RT and poured onto water. Extraction with EtOAc was followed by washing the organic layer with 1 M aqueous HCl, sat. aqueous $NaHCO_3$ solution and brine. After drying over Na_2SO_4 and removal of solvent, purification by FCC (hexane / EtOAc 3:1) afforded the title compound. ^1H NMR (d_6-DMSO, 400 MHz): δ 1.06 (t, J = 8 Hz, 3H), 3.98 (q, J = 8 Hz, 2H), 4.24 (s, 2H), 7.66 (d, J = 10 Hz, 1H), 7.79 (d, J = 10 Hz, 1H), 7.96 (d, J = 10 Hz, 1H), 8.13 (d, J = 10 Hz, 1H), 8.54 (s, 1H).

Preparation of (2-Chloro-6-trifluoromethanesulfonyloxy-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-6-hydroxy-naphthalen-1-yl)-acetic acid ethyl ester (3.39 g, 12.80 mmol) was dissolved under an atmosphere of argon in pyridine (35 ml). After cooling to 0 °C, trifluoromethanesulfonic acid anhydride (2.32 ml, 14.08 mmol) was added dropwise during 15 minutes. After stirring at 0°C for 15 minutes and at RT for 1 h, TLC analysis indicates complete consumption of starting material. The reaction mixture was poured into 1 M aqueous $NaHCO_3$ solution. After extraction with EtOAc, washing with brine and drying of the organic layer over Na_2SO_4, concentration yielded the crude reaction product. Purification by FCC (hexane / EtOAc 4:1) afforded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.48 (t, J = 9 Hz, 3H), 4.41 (q, J = 9 Hz, 2H), 4.52 (s, 2H), 7.68 (d, J = 10 Hz, 1H), 7.82 (d, J = 10 Hz, 1H), 7.98 - 8.00 (m, 2H), 8.27 (d, J = 10 Hz, 1H).

Preparation of (2-Chloro-6-hydroxy-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-6-methoxy-naphthalen-1-yl)-acetic acid ethyl ester (5.43 g, 19.48 mmol) and tetrabutylammonium iodide (9.35 g, 25.32 mmol) were dissolved under an atmosphere of argon in CH_2Cl_2 (110 ml). The reaction mixture was cooled to -78 °C and a 1 M solution of BBr_3 in CH2Cl2 (48.7 ml, 48.7 mmol) was added during 15 minutes. After stirring at -78 °C for 10 minutes and at RT for 10 minutes, TLC analysis indicates complete consumption of starting material. The reaction mixture was poured on conc. aqueous $NaHCO_3$ solution, and

the mixture was vigorously stirred for 20 minutes at RT. After extraction with CH_2Cl_2, the organic layer was washed with brine and dried over Na_2SO_4. Purification by FCC (hexane / EtOAc 2:1) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.19 (t, J = 9 Hz, 3H), 4.12 (q, J = 9 Hz, 2H), 4.18 (s, 2H), 5.35 – 5.60 (br, 1H), 6.93 (s, 1H), 6.99 (d, J = 10 Hz, 1H), 7.33 (d, J = 10 Hz, 1H), 7.42 (d, J = 10 Hz, 1H), 7.70 (d, J = 10 Hz, 1H). ES^+-MS: 265.2, 266.8 $[M + H]^+$.

Preparation of (2-Chloro-6-methoxy-naphthalen-1-yl)-acetic acid ethyl ester

A mixture of (2-Chloro-6-methoxy-naphthalen-1-yl)-acetic acid ethyl ester and (2-chloro-6-methoxy-3,4-dihydro-naphthalen-1-yl)-acetic acid ethyl ester (4.07 g, approx. 14.6 mmol) was dissolved under an atmosphere of argon in dioxane (40 ml). 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ, 7.30 g, 32 mmol) was added, and the reaction mixture was refluxed for 4 h. After cooling to RT, addition of MeOH renders the reaction mixture homogeneous. Silica gel was added, and the solvent was removed by rotary evaporation. Purification by FCC (hexane / EtOAc 980:20 to 960:40) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.32 (t, J = 9 Hz, 3H), 4.00 (s, 3H), 4.26 (q, J = 9 Hz, 3H), 4.34 (s, 2H), 7.21 (s, 1H), 7.30 (d, J = 10 Hz, 1H), 7.52 (d, J = 10 Hz, 1H), 7.71 (d, J = 10 Hz, 1H), 7.92 (d, J = 10 Hz, 1H). ES^+-MS: 279.1, 280.9 $[M + H]^+$.

Preparation of (2-Chloro-6-methoxy-naphthalen-1-yl)-acetic acid ethyl ester and (2-Chloro-6-methoxy-3,4-dihydro-naphthalen-1-yl)-acetic acid ethyl ester

A mixture of (2-chloro-1-hydroxy-6-methoxy-1,2,3,4-tetrahydro-naphthalen-1-yl)-acetic acid ethyl ester (5.0 g, 16.64 mmol), 1,1-diphenyl ethene (3.2 ml), 1-methyl-naphthalene (3 ml) and palladium on charcoal (10%, 500 mg) was heated under an atmosphere of argon to 180 °C. After 3 h, TLC analysis indicates complete consumption of starting material. The reaction mixture was cooled to RT, diluted with EtOAc and filtered. Removal of EtOAc and purification by FCC (hexane 100 to hexane/EtOAc 980:20 to 960:40) afforded the title compound mixture.

Preparation of (2-Chloro-1-hydroxy-6-methoxy-1,2,3,4-tetrahydro-naphthalen-1-yl)-acetic acid ethyl ester

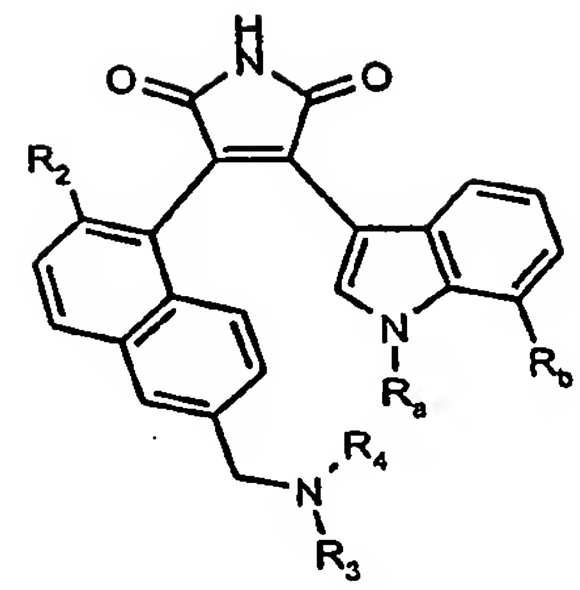
A solution of EtOAc (7.2 ml, 73.96 mmol) in THF (20 ml) was slowly added under an atmosphere of argon at -78 °C to a solution of lithium diisopropylamine (prepared from 10.5 ml of diisopropylamine (73.96 mmol) and 46.2 ml of 1.6 M n-BuLi in hexane (73.96 mmol)) in

THF (20 ml). After stirring at -78 °C for 30 minutes, a solution of 2-chloro-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (7.79 g, 36.98 mmol) in THF (20 ml) was slowly added during 30 minutes. The reaction mixture was stirred at -78 °C for 24 h. TLC analysis indicates complete conversion of starting material. The reaction mixture was diluted with EtOAc and poured into a sat. aqueous solution of NH_4Cl. The organic layer was separated and washed with brine. After drying over Na_2SO_4, the solvent was removed. Purification by FCC (hexane / EtOAc 920:80 to 880:120) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.22 (t, J = 9 Hz, 3H), 2.33 – 2.41 (m, 2H), 2.80 – 3.12 (m, 4H); 3.12 (s, 1H), 3.78 (s, 3H), 4.12 (q, J = 9 Hz, 2H), 5.01 – 5.04 (m, 1H), 6.60 – 6.62 (m, 1H), 6.78 – 6.82 (m, 1H), 7.52 (d, J = 10 Hz, 1H).

Preparation of 2-Chloro-6-methoxy-3,4-dihydro-2H-naphthalen-1-one

A solution of 6-Methoxy-3,4-dihydro-2H-naphthalen-1-one (5.0 g, 28.37 mmol) in THF (25 ml) was slowly added under an atmosphere of argon at -78 °C to a solution of lithium diisopropyl amine in THF (25 ml; prepared from 4.0 ml of diisopropylamine (28.37 mmol) and 17.7 ml of 1.6 M n-BuLi in hexane (28.37 mmol)). After 30 minutes at -78 °C, a solution of para-tolylsulfonyl chloride (5.41 g, 28.37 mmol) in THF (25 ml) was added during 20 minutes. The dry ice cooling bath was removed, and the reaction mixture was allowed to reach RT. After 1 h, TLC analysis indicates complete consumption of starting material. A sat. aqueous solution of NH_4Cl (100 ml) was added, and the mixture was stirred at RT for 15 minutes. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated. Purification by FCC (hexane / EtOAc 920 : 80 to 880 : 120) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 2.54 – 2.63 (m, 1H), 2.68 – 2.75 (m, 1H), 3.04 – 3.12 (m, 1H), 3.38 – 3.46 (m, 1H), 4.02 (s, 3H); 4.72 – 4.76 (m, 1H), 6.87 (s, 1H), 7.00 – 7.04 (m, 1H), 8.22 (d, J = 10 Hz, 1H). ES^+-MS: 279.1, 280.9 $[M + H]^+$.

By following the procedure of Example 1, but using the appropriate starting materials, the compounds of formula A wherein R_a, R_b, R_2 to R_4 are as indicated in Table 1 below, may be obtained.

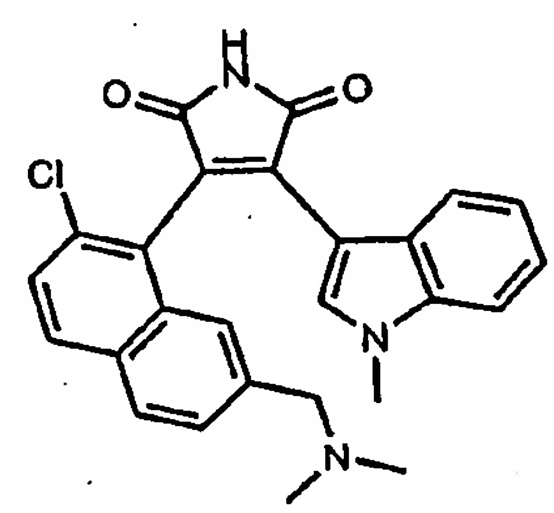


A

Table 1

	R_2	R_3	R_4	R_a	R_b	MS
2.	H	CH_3	CH_3	H	H	MH^+ 430
3.	H	CH_3	CH_3	H	CH_3	MH^+ 444
4.	H	CH_3	H	H	H	MH^+ 416
5.	H	CH_3	H	H	CH_3	MH^+ 430
6.	Cl	H	H	H	CH_3	MH^+ 382
7.	Cl	H	H	H	H	MH^+ 368
8.	Cl	H	H	CH_3	H	MH^+ 382

Example 9: 3-(2-Chloro-7-dimethylaminomethyl-naphthalen-1-yl)-4-(1-methyl-1H-indol-3-yl)-pyrrole-2,5-dione



Activated 3Å molecular sieve (2.0 g) was added to a solution of 2-(2-Chloro-7-dimethylaminomethyl-naphthalen-1-yl)-acetamide (1.0 g, 3.61 mmol) and (1-Methyl-1H-indol-3-yl)-oxo-acetic acid methyl ester (1.02 g, 4.69 mmol) in dry THF (50 ml) under an atmosphere of argon. A solution of 1.0 M KOtBu in THF (10.9 ml, 10.9 mmol) was then added in one portion at RT. After 1 h at RT, TLC analysis indicates complete conversion of starting materials. The reaction mixture was diluted with EtOAc and poured into a saturated aqueous NH_4Cl solution. The organic layer was separated, washed with brine, dried over Na_2SO_4, and the organic solvent was evaporated. The residue was purified by FCC (EtOAc /

AcOH / H_2O 600:150:150) to afford the title compound, which was dissolved in glacial AcOH and lyophilized. The title compound was obtained as the water soluble bis-acetate salt. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 1.80 (s, 6H), 3.20 – 3.42 (m, 2H), 6.10 (d, J = 9.0 Hz, 1H), 6.44 (t, J = 9.0 Hz, 1H), 6.94 (t, J = 9.0 Hz, 1H), 7.31 (d, J = 9.0 Hz, 1H), 7.34 (d, J = 9.0 Hz, 1H), 7.42 (s, 1H), 7.63 (d, J = 10.8 Hz, 1H), 7.89 (d, J = 9.6 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H), 8.10 (s, 1H), 11.0 – 11.3 (br, 1H). ES$^+$-MS: 444, 446 $[M + H]^+$.

Preparation of 2-(2-Chloro-7-dimethylaminomethyl-naphthalen-1-yl)-acetamide

(2-Chloro-7-dimethylaminomethyl-naphthalen-1-yl)-acetic acid ethyl ester (2.70 g, 8.82 mmol) and formamide (1.17 ml, 29.57 mmol) are dissolved under an atmosphere of argon in dry DMF (25 ml). The solution was heated to 105 °C, and NaOMe (1.64 ml of a 5.4 M solution in MeOH, 8.82 mmol) was added dropwise during 10 minutes. After 1 hour at 105°C, TLC analysis indicates complete consumption of starting material. The reaction mixture was cooled to RT, diluted with water, and adjusted to a pH of 6 – 7 by the addition of 1 M $NaHSO_4$ solution. The mixture was concentrated and purified by FCC (CH_2Cl_2 / EtOH / NH_3 conc. 90:9:1) to yield the title compound. ^{1}H NMR (d_6-DMSO, 400 MHz): δ 2.01 (s, 6H), 3.48 (s, 3H), 4.02 (s, 3H), 6.9 – 7.0 (br, 1H), 7.41 – 7.47 (m, 2H); 7.47 – 7.85 (br, 1H), 7.75 – 7.88 (m, 2H), 7.80 (d, J = 12.1 Hz, 1H). ES$^+$-MS: 277.3, 279.2 $[M + H]^+$.

Preparation of (2-Chloro-7-dimethylaminomethyl-naphthalen-1-yl)-acetic acid ethyl ester

Dimethylamine (5.6 M solution in EtOH, 4.2 ml, 23.20 mmol) was added under an atmosphere of argon to a solution of (2-Chloro-7-formyl-naphthalen-1-yl)-acetic acid ethyl ester (4.28 g, 15.46 mmol) in THF (80 ml). The mixture was stirred at RT for 18 h, before a solution of sodium cyanoborohydride (1.16 g, 18.56 mmol) in MeOH (20 ml) and glacial acetic acid (4.4 ml, 77.33 mmol) were added. After stirring at RT for 2 h, TLC analysis indicates complete consumption of starting material. The reaction mixture was diluted with water and adjusted to pH 8 – 9 by the addition of conc. aq. $NaHCO_3$ solution. Extraction with EtOAc, washing with brine, drying over Na_2SO_4 and removal of solvent yielded the crude reaction product. Purification by FCC (EtOAc, then EtOAc / MeOH 4:1) afforded the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, J = 8.8 Hz, 3H), 2.28 (s, 6H), 3.62 (s, 3H), 4.18 (q, J = 8.8 Hz, 2H), 4.32 (s, 3H), 7.48 (d, J = 9.9 Hz, 1H), 7.52 (d, J = 9.9 Hz, 1H), 7.73 (d, J = 9.9 Hz, 1H), 7.80 – 7.84 (m, 2H). ES$^+$-MS: 306.3, 308.2 $[M + H]^+$.

Preparation of (2-Chloro-7-formyl-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-7-cyano-naphthalen-1-yl)-acetic acid ethyl ester (5.53 g, 20.20 mmol) was dissolved in a mixture of water (70 ml), pyridine (130 ml) and glacial acetic acid (70 ml). Sodium hypophosphite (17.13 g, 161.62 mmol) and Raney nickel (13 g) were added at RT. The reaction mixture was heated to 100°C for 1 h. TLC analysis indicates complete consumption of starting material. The reaction mixture was cooled to RT, filtered through Celite and concentrated on a rotary evaporator. The residue was taken up in 2 M aqueous HCl. Extraction with EtOAc, removal of solvent and purification by FCC (hexane / EtOAc 5:1) yielded the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.28 (t, J = 8.8 Hz, 3H), 4.22 (q, J = 8.8 Hz, 2H), 4.39 (s, 2H), 7.68 (d, J = 9.9 Hz, 1H), 7.83 (d, J = 9.9 Hz, 1H), 7.95 – 8.03 (m, 2H), 8.48 (s, 1H), 10.2 (s, 1H). ES$^-$-MS: 275.3, 277.3 [M + H]$^+$.

Preparation of (2-Chloro-7-cyano-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-7-trifluoromethanesulfonyloxy-naphthalen-1-yl)-acetic acid ethyl ester (9.30 g, 23.43 mmol) was dissolved in DMF (80 ml) under an atmosphere of argon. Palladium(0) tetrakis(triphenylphosphane) (1.08 g, 0.9375 mmol) and zinc(II) cyanide (5.50 g, 46.87 mmol) were added. The reaction mixture was heated to 125 °C. After 1 h, TLC analysis indicates complete consumption of starting material. The suspension was cooled to RT and poured onto water. After stirring for 15 minutes, filtration and concentration afforded the crude reaction product. Purification by FCC (hexane / EtOAc 4 : 1) afforded the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, J = 8.8 Hz, 3H), 4.19 (q, J = 8.8 Hz, 2 H), 4.28 (s, 2H), 7.62 – 7.66 (m, 2H), 7.79 (d, J = 9.9 Hz, 1H), 7.92 (d, J = 9.9 Hz, 1H), 8.32 (s, 1H). ES$^+$-MS: 274.2 [M + H]$^+$.

Preparation of (2-Chloro-7-trifluoromethanesulfonyloxy-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-7-hydroxy-naphthalen-1-yl)-acetic acid ethyl ester (8.03 g, 30.33 mmol) was dissolved under an atmosphere of argon in pyridine (60 ml). After cooling to 0°C, trifluoromethanesulfonic acid anhydride (5.50 ml, 33.36 mmol) was added dropwise during 15 minutes. After stirring at 0°C for 15 minutes and at RT for 1 h, TLC analysis indicated complete consumption of starting material. The reaction mixture was poured into 1 M aqueous $NaHCO_3$ solution. After extraction with EtOAc, washing with brine and drying of the organic layer over Na_2SO_4, concentration yielded the crude reaction product. Purification by FCC (hexane / EtOAc 4:1) afforded the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.13 (t, J = 9.4 Hz, 3H), 4.08 (q, J = 9.4 Hz, 2H), 4.15 (s, 2H), 7.28 – 7.30 (m, 1H), 7.48 (d, J = 11

Hz, 1H), 7.69 (d, J = 11 Hz, 1H), 7.72 (m, 1H), 7.82 (d, J = 11 Hz, 1H). ES^+-MS: 414.2, 416.0, 397.1 $[M + H]^+$.

Preparation of (2-Chloro-7-hydroxy-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-7-methoxy-naphthalen-1-yl)-acetic acid ethyl ester (12.0 g, 43.10 mmol) and tetrabutylammonium iodide (20.7 g, 56.04 mmol) were dissolved under an atmosphere of argon in CH_2Cl_2 (240 ml). The reaction mixture was cooled to -78 °C and a 1 M solution of BBr_3 in CH_2Cl_2 (108 ml, 107.77 mmol) was added during 30 minutes. After stirring at -78 °C for 15 minutes and at RT for 1 h, TLC analysis indicates complete consumption of starting material. A sat. aqueous solution of $NaHCO_3$ (8 ml) was carefully added. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated. Purification by FCC (hexane / EtOAc 4:1 to 3:2) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.51 (t, J = 9.9 Hz, 3H), 4.43 (q, J = 9.9 Hz, 2H), 4.48 (s, 2H), 6.28 – 6.36 (br, 1H), 7.29 – 7.32 (m, 1H), 7.48 – 7.49 (m, 1H), 7.58 (d, J = 10 Hz, 1H), 7.89 (d, J = 10 Hz, 1H), 7.96 (d, J = 10 Hz, 1H). ES^+-MS: 265.2, 267.2 $[M + H]^+$.

Preparation of (2-Chloro-7-methoxy-naphthalen-1-yl)-acetic acid ethyl ester

A mixture of [2-Chloro-7-methoxy-3,4-dihydro-2H-naphthalen-(1E/Z)-ylidene]-acetic acid ethyl ester and of (2-Chloro-7-methoxy-3,4-dihydro-naphthalen-1-yl)-acetic acid ethyl ester (26.82 g, 95.52 mmol) was dissolved under an atmosphere of argon in dioxane (280 ml). 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ, 47.70 g, 210.16 mmol) was added, and the reaction mixture was refluxed for 2 h. TLC analysis indicates complete conversion of starting material. After cooling to RT, addition of MeOH renders the reaction mixture homogeneous. Silica gel (250 g) was added, and the solvent was removed by rotary evaporation. Purification by FCC (hexane / EtOAc 9:1) yielded the title compound. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.24 (t, J = 8.8 Hz, 3H), 3.95 (s, 3H), 4.19 (q, J = 8.8 Hz, 2H), 4.28 (s, 2H), 7.16 – 7.19 (m, 1H), 7.22 (s, 1H), 7.38 (d, J = 10 Hz, 1H), 7.68 (d, J = 10 Hz, 1H), 7.75 (d, J = 10 Hz, 1H). ES^+-MS: 279.2, 281.2 $[M + H]^+$.

Preparation of (2-Chloro-7-methoxy-3,4-dihydro-naphthalen-1-yl)-acetic acid ethyl ester

(2-Chloro-1-hydroxy-7-methoxy-1,2,3,4-tetrahydro-naphthalen-1-yl)-acetic acid ethyl ester (42.7 g, 142.9 mmol) was dissolved under an atmosphere of argon in pyridine (250 ml). Trifluoromethanesulfonic acid anhydride (30.7 ml, 185.8 mmol) was added during 30 minutes, while keeping the temperature at 25 °C with occasional cooling with a ice bath.

After addition was complete, the reaction mixture was warmed to 50 °C for 2 h. TLC analysis indicates complete conversion of starting material. 2 M aqueous HCl (100 ml) was carefully added, and then the reaction mixture was concentrated to dryness on the rotary evaporator. The residue was taken up in 2 M aqueous HCl (100 ml) and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated. Purification by FCC (EtOAc) afforded the title compounds. ES^+-MS: 281.2, 283.2 $[M + H]^+$.

Preparation of (2-Chloro-1-hydroxy-7-methoxy-1,2,3,4-tetrahydro-naphthalen-1-yl)-acetic acid ethyl ester

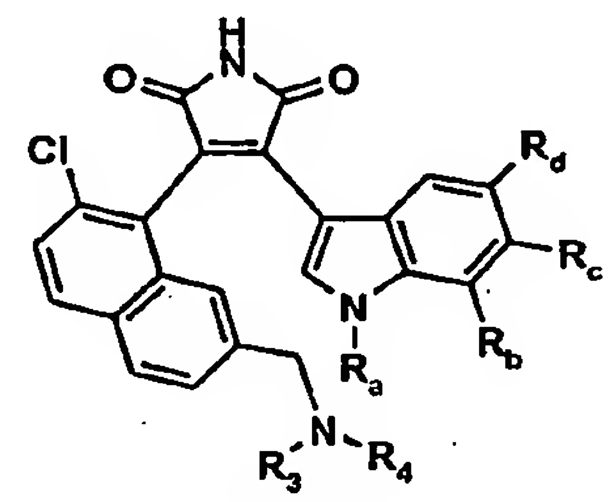
A solution of EtOAc (16.1 ml, 164.48 mmol) in THF (250 ml) was slowly added under an atmosphere of argon at -78 °C to a solution of lithium diisopropylamine (prepared from 23.3 ml of diisopropylamine (164.48 mmol) and 102.8 ml of 1.6 M n-BuLi in hexane (164.48 mmol) in THF (250 ml). After stirring at -78 °C for 30 minutes, a solution of 2-chloro-7-methoxy-3,4-dihydro-2H-naphthalen-1-one (31.5 g, 149.53 mmol) in THF (250 ml) was slowly added during 30 minutes. The reaction mixture was stirred at -78 °C for 1 h. TLC analysis indicates complete conversion of starting material. A sat. aqueous solution of NH_4Cl (250 ml) was carefully added to the reaction mixture at -78 °C. The mixture was warmed to RT. The organic layer was separated, diluted with EtOAc and washed with brine. After drying over Na_2SO_4, the solvent was removed. Purification by FCC (hexane / EtOAc 4:1) yielded the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (t, J = 9.4 Hz, 3H), 2.32 – 2.48 (m, 2H), 2.78 – 2.88 (m, 1H), 2.86 – 3.02 (m, 2H), 3.05 – 3.14 (m, 1H), 3.82 (s, 3H), 4.18 (q, J = 9.4 Hz, 2H), 5.02 – 5.08 (m, 1H), 6.81 – 6.84 (m, 1H), 7.03 (d, J = 10.5 Hz, 1H), 7.18 – 7.19 (m, 1H). ES^+-MS: 281.3, 283.3 $[M + H – H_2O]^+$.

Preparation of 2-Chloro-7-methoxy-3,4-dihydro-2H-naphthalen-1-one

A solution of 7-Methoxy-3,4-dihydro-2H-naphthalen-1-one (25.6 g, 145.28 mmol) in THF (300 ml) was slowly added under an atmosphere of argon at -78 °C to a solution of lithium diisopropyl amine in THF (300 ml; prepared from 22.6 ml of diisopropylamine (160 mmol) and 100 ml of 1.6 M n-BuLi in hexane (160 mmol)). After 30 minutes at -78 °C, a solution of para-tolylsulfonyl chloride (30.5 g, 159.8 mmol) in THF (300 ml) was added during 20 minutes. The dry ice cooling bath was removed, and the reaction mixture was allowed to reach RT. After 1 h, TLC analysis indicates complete consumption of starting material. A sat. aqueous solution of NH_4Cl (100 ml) was added, and the mixture was stirred at RT for 15 minutes. The organic layer was separated, washed with brine, dried over Na_2SO_4 and

concentrated. Purification by FCC (hexane / EtOAc 3:1) yielded the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.32 – 2.52 (m, 2H), 2.82 – 2.90 (m, 2H), 3.10 – 3.18 (m, 2H), 3.78 (s, 1H), 4.52 – 4.58 (m, 1H), 7.01 – 7.05 (m, 1H), 7.11 (d, J = 8.8 Hz, 1H), 7.47 – 7.48 (m, 1H). ES^+-MS: 211.3, 213.3 $[M + H]^+$.

By following the procedure of Example 9, but using the appropriate starting materials, the compounds of formula A wherein R_a, R_b, R_3 and R_4 are as indicated in Table 2 below, may be obtained.



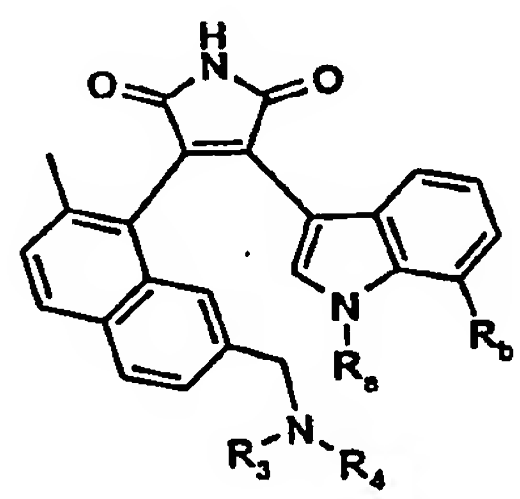
A

Table 2

	R_3	R_4	R_a	R_b	R_c	R_d	MS
10.	H	H	CH_3	H	H	H	MH^+ 416
11.	H	CH_3	H	H	H	H	MH^+ 513
12.	H	CH_3	CH_3	H	H	H	MH^+ 430
13.	H	CH_3	H	CH_3	H	H	MH^+ 444
14.	H	CH_3	H	H	CH_3	H	MH^+ 444
15.	H	CH_3	H	H	H	CH_3	MH^+ 444
16.	CH_3	CH_3	H	CH_3	H	H	MH^+ 444
17.	CH_3	CH_3	H	H	H	H	MH^+ 431
18.	CH_3	CH_3	H	H	CH_3	H	MH^+ 445
19.	CH_3	CH_3	H	H	H	CH_3	MH^+ 445
20.	CH_3	CH_2CH_3	CH_3	H	H	H	MH^+ 459
21.	CH_2CH_3	CH_2CH_3	CH_3	H	H	H	MH^+ 473
22.	H	CH_2CH_3	CH_3	H	H	H	MH^+ 445
23.	H	i-propyl	CH_3	H	H	H	MH^+ 459
24.	$-CH_2-CH_2-N(CH_3)-CH_2-CH_2-$		CH_3	H	H	H	MH^+ 500
25.	$-CH_2-CH_2-CH_2-$		CH_3	H	H	H	MH^+ 471

	CH_2-					

By following the procedure of Example 9, but using the appropriate starting materials, the compounds of formula B wherein R_a, R_b, R_3 and R_4 are as indicated in Table 3 below, may be obtained.

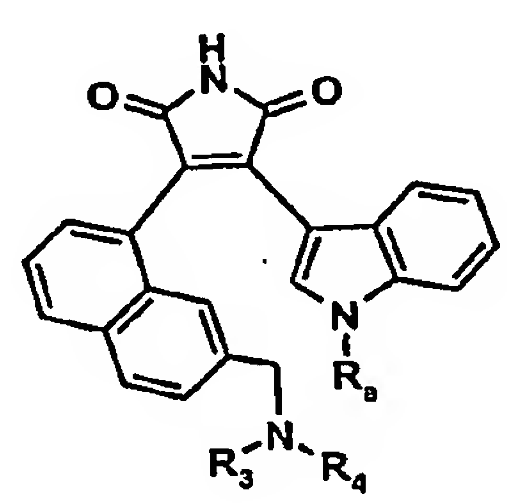


B

Table 3

	R_3	R_4	R_a	R_b	MS
26.	H	H	CH_3	CH_3	MH^+ 410
27.	H	H	H	CH_3	MH^+ 396
28.	H	H	H	H	MH^+ 382
29.	H	H	CH_3	H	MH^+ 396

By following the procedure of Example 9, but using the appropriate starting materials, the compounds of formula B wherein R_a, R_3 and R_4 are as indicated in Table 4 below, may be obtained.



B

Table 4

	R_3	R_4	R_a	MS
30.	H	H	H	MH^+ 368
31.	H	H	CH_3	MH^+ 382

32.	H	H	CH_3	MH^+ 368
33.	H	H	H	MH^+ 354

The compounds of formula I in free form or in pharmaceutically acceptable salt form exhibit valuable pharmacological properties, e.g. inhibiting Protein Kinase C (PKC), e.g. PKC isoforms like α, β, δ, ε, η or θ activity, inhibiting T-cell activation and proliferation, e.g. by inhibiting production by T-cells or cytokines, e.g. IL-2, by inhibiting the proliferative response of T-cells to cytokines, e.g. IL-2, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. <u>In vitro</u>

1. <u>Protein Kinase C assay</u>

The compounds of formula I are tested for their activity on different PKC isoforms according to a published method (D. Geiges et al. Biochem. Pharmacol. 1997;53:865-875) The assay is performed in a 96-well polypropylene microtiterplate (Costar 3794) that has been previously siliconized with Sigmacote (Sigma SL-2). The reaction mixture (50 µl) contains 10 µl of the relevant PKC isozyme together with 25 µl of the test compound and 15 µl of a mix solution that contains 200 µg/ml protamine sulfate, 10 mM $Mg(NO_3)_2$, 10 µM ATP (Boehringer 519987) and 3750 Bq of ^{33}P-ATP (Hartmann Analytic SFC301, 110TBq/mmol) in 20 mM Tris-buffer pH 7.4 + 0.1% BSA. Incubation is performed for 15 min at 32°C in a microtiterplate shaking incubator (Biolabo Scientific Instruments). Reaction is stopped by adding 10 µl of 0.5 M Na_2EDTA, pH 7.4. 50 µl of mixture are pipetted onto a pre-wetted phosphocellulose paper (Whatmann 3698-915) under gentle pressure. Non-incorporated ATP is washed away with 100 µl bi-dist H_2O. The paper is washed twice in 0.5% H_3PO_4 for 15 min followed by 5 min in EtOH. Thereafter the paper is dryed and placed in an omnifilter (Packard 6005219), and overlayed with 10 µl/well of Microscint-O (Packard 6013611) before counting in a Topcount radioactivity counter (Packard). IC_{50} measurement is performed on a routine basis by incubating a serial dilution of inhibitor at concentrations ranging between 1-1000 µM according to the method described above. IC_{50} value are calculated from the graph by sigmoidal curve fitting.

2. <u>Protein Kinase C θ Assay</u>

Human recombinant PKCθ is used under the assay conditions as described above. In this assay, compounds of formula I inhibit PKC θ with an $IC_{50} \leq 1$ µM. For example, compound of

Example 3 inhibits PKCθ with an IC_{50} of 12.8 nM and compound of Example 5 with an IC_{50} of 28.1 nM.

3. Protein Kinase Cα Assay

Human recombinant PKCα was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCα with an $IC_{50} \leq 1$ μM. For example, compound of Example 3 inhibits PKCα with an IC_{50} of 12.2 nM, compound of Example 5 with an IC_{50} of 17.8 nM, and compound of Example 9 with an IC_{50} of 21 nM.

4. Protein Kinase Cβ1 Assay

Human recombinant PKCβ1 was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCβ1 with an $IC_{50} \leq 1$ μM. For example, compound of Example 9 inhibits PKCβ1 with an IC_{50} of 56 nM.

5. Protein Kinase Cδ Assay

Human recombinant PKCδ was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCδ with an $IC_{50} \leq 1$ μM. For example, compound of Example 3 inhibits PKCδ with an IC_{50} of 29.0 nM and compound of Example 5 with an IC_{50} of 33.7 nM.

6. Protein Kinase Cε Assay

Human recombinant PKCε was obtained from Oxford Biomedical Research and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCε with an $IC_{50} \leq 1$ μM. For example, compound of Example 3 inhibits PKCδ with an IC_{50} of 16.6 nM and compound of Example 5 with an IC_{50} of 21.8 nM.

7. Protein Kinase Cη Assay

Human recombinant PKCη was obtained from PanVera and is used under the assay conditions as described under Section A.1 above. In this assay, compounds of formula I inhibit PKCη with an $IC_{50} \leq 1$ μM. For example, compound of Example 3 inhibits PKCδ with an IC_{50} of 47.2 nM and compound of Example 5 with an IC_{50} of 34.8 nM.

8. CD28 costimulation assay

The assay is performed with Jurkat cells transfected with a human interleukin-2 promoter/reporter gene construct as described by Baumann G et al. in Transplant. Proc. 1992;24:43-8, the ß-galactosidase reporter gene being replaced by the luciferase gene (de Wet J., et al., Mol. Cell Biol. 1987, 7(2), 725-737). Cells are stimulated by solid phase-coupled antibodies or phorbol myristate acetate (PMA) and the Ca^{++} ionophore ionomycin as follows. For antibody-mediated stimulation Microlite TM1 microtiter plates (Dynatech) are coated with 3 µg/ml goat anti-mouse IgG Fc antibodies (Jackson) in 55 µl phosphate-buffered saline (PBS) per well for three hours at RT. Plates are blocked after removing the antibodies by incubation with 2% bovine serum albumin (BSA) in PBS (300 µl per well) for 2 hours at RT. After washing three times with 300 µl PBS per well, 10 ng/ml anti-T cell receptor antibodies (WT31, Becton & Dickinson) and 300 ng/ml anti-CD28 antibodies (15E8) in 50 µl 2% BSA/PBS are added as stimulating antibodies and incubated overnight at 4°C. Finally the plates are washed three times with 300 µl PBS per well. Seven three-fold serial dilutions of test compounds in duplicates in assay medium (RPMI 1640/10% fetal calf serum (FCS) containing 50 µM 2-mercaptoethanol, 100 units/ml penicillin and 100 µg/ml streptomycin) are prepared in separate plates, mixed with transfected Jurkat cells (clone K22 290_H23) and incubated for 30 minutes at 37°C in 5% CO_2. 100 µl of this mixture containing 1×10^5 cells are then transferred to the antibody-coated assay plates. In parallel 100 µl are incubated with 40 ng/ml PMA and 2 µM ionomycin. After incubation for 5.5 hours at 37°C in 5% CO_2, the level of luciferase is determined by bioluminescence measurement. The plates are centrifuged for 10 min at 500 g and the supernatant is removed by flicking. Lysis buffer containing 25 mM Tris-phosphate, pH 7.8, 2 mM DTT, 2 mM 1.2-diaminocyclohexane-N,N,N',N-tetraacetic acid, 10 % (v/v) glycerol and 1 % (v/v) Triton X-100 is added (20 µl per well). The plates are incubated at RT for 10 minutes under constant shaking. Luciferase activity is assessed with a bioluminescence reader (Labsystem, Helsinki, Finland) after automatic addition of 50 µl per well luciferase reaction buffer containing 20 mM Tricine, 1.07 mM $(MgCO_3)_4Mg(OH)_2x5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM DTT, 270 µM coenzyme A, 470 µM luciferin (Chemie Brunschwig AG), 530 µM ATP, pH 7.8. Lag time is 0.5 seconds, total measuring time is 1 or 2 seconds. Low control values are light units from anti-T cell receptor- or PMA-stimulated cells, high controls are from anti-T cell receptor/anti-CD28- or PMA/ionomycin-stimulated cells without any test sample. Low controls are subtracted from all values. The inhibition obtained in the presence of a test compound is calculated as percent inhibition of the high control. The concentration of test compounds resulting in 50% inhibition (IC_{50}) is determined from the dose-response curves. In this assay,

compounds of formula I inhibit anti-T cell receptor/anti-CD28 and PMA/ionomycin stimulated Jurkat cells with an $IC_{50} \leq 1$ µM.
For example, compound of Example 3 has an IC_{50} of 87.5 nM and compound of Example 9 has an IC_{50} of 296 nM.

9. Allogeneic Mixed Lymphocyte Reaction (MLR)

The two-way MLR is performed according to standard procedures (J. Immunol. Methods, 1973, 2, 279 and Meo T. et al., Immunological Methods, New York, Academic Press, 1979, 227-39). Briefly, spleen cells from CBA and BALB/c mice (1.6×10^5 cells from each strain per well in flat bottom tissue culture microtiter plates, 3.2×10^5 in total) are incubated in RPMI medium containing 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco BRL, Basel, Switzerland), 50 µM 2-mercaptoethanol (Fluka, Buchs, Switzerland) and serially diluted compounds. Seven three-fold dilution steps in duplicates per test compound are performed. After four days of incubation 1 µCi ^{3}H-thymidine is added. Cells are harvested after an additional five-hour incubation period, and incorporated ^{3}H-thymidine is determined according to standard procedures. Background values (low control) of the MLR are the proliferation of BALB/c cells alone. Low controls are subtracted from all values. High controls without any sample are taken as 100% proliferation. Percent inhibition by the samples is calculated, and the concentrations required for 50% inhibition (IC_{50} values) are determined. For example, compound of Example 3 has an IC_{50} of 182.5 nM and compound of Example 9 has an IC_{50} of 144 nM.

10. Inhibition of GSK-3β

The GSK-3β bibding assay is performed in 50µl reactions in 96 well polypropylene plate, each reaction containing 20mM magnesium chloride, 40 µM ATP, 2mM DTT, 88.5µM biotinylated and phosphorylated CREB-peptide substrate (biotin-KRREILSRRPS(PO_4)YR-OH ; Q. M. Wang et al., J. Biol. Chem. 269, 14566-14574, 1994), [γ-^{33}P]ATP (1µCi) and 2µl of the compound to be tested in DMSO(various concentrations). 15µl of GSK-3β (various concentrations) is added and the mixture is incubated at 30°C for 1 hour. The reaction is stopped by transferring 25 µl of the mixture to a phosphocellulose plate containing 130µl of 1.85% phosphoric acid. The free radionucleotides in the membrane are washed off under vacuum with 1.85% phosphoric acid (5 times). After the last wash, the plate is transferred to an adaptor plate and 50µl of scintillation cocktail (Microscint-20, Packard, cat. # 20-133) is added to each well and the amount of radioactivity is counted in a top counter. Compounds of formula I are active in this assay.

B. In vivo

Rat Heart transplantation

The strain combination used: Male Lewis (RT[1] haplotype) and BN (RT[1] haplotype). The animals are anaesthetised using inhalational isofluorane. Following heparinisation of the donor rat through the abdominal inferior vena cava with simultaneous exsanguination via the aorta, the chest is opened and the heart rapidly cooled. The aorta is ligated and divided distal to the first branch and the brachiocephalic trunk is divided at the first bifurcation. The left pulmonary artery is ligated and divided and the right side divided but left open. All other vessels are dissected free, ligated and divided and the donor heart is removed into iced saline.

The recipient is prepared by dissection and cross-clamping of the infra-renal abdominal aorta and vena cava. The graft is implanted with end-to-side anastomoses, using 10/0 monofilament suture, between the donor brachiocephalic trunk and the recipient aorta and the donor right pulmonary artery to the recipient vena cava. The clamps are removed, the graft tethered retroabdominally, the abdominal contents washed with warm saline and the animal is closed and allowed to recover under a heating lamp. Graft survival is monitored by daily palpation of the beating donor heart through the abdominal wall. Rejection is considered to be complete when heart beat stops. Increases of graft survival are obtained in animals treated with a compound of formula I administered orally at a daily dose of 1 to 30 mg/kg bid.

Graft v. Host Model

Spleen cells (2×10^7) from Wistar/F rats are injected subcutaneously into the right hind footpad of (Wistar/F x Fischer 344)F_1 hybrid rats. The left footpad is left untreated. The animals are treated with the test compounds on 4 consecutive days (0-3). The popliteal lymph nodes are removed on day 7, and the weight differences between two corresponding lymph nodes are determined. The results are expressed as the inhibition of lymph node enlargement (given in percent) comparing the lymph node weight differences in the experimental groups to the weight difference between the corresponding lymph nodes from a group of animals left untreated with a test compound.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by T lymphocytes and/or PKC, e.g. acute or chronic rejection of organ or tissue allo- or xenografts, graft versus host diseases, atherosclerosis, vascular occlusion due to vascular injury such as angioplasty, restenosis, obesity, syndrome X,

impaired glucose tolerance, polycystic ovary syndrome, hypertension, heart failure, chronic obstructive pulmonary disease, CNS diseases such as Alzheimer disease or amyotrophic lateral sclerosis, cancer, infectious diseases such as AIDS, septic shock or adult respiratory distress syndrome, ischemia/reperfusion injury e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, or traumatic shock, e.g. traumatic brain injury. The compounds of formula I are also useful in the treatment and/or prevention of T-cell mediated acute or chronic inflammatory diseases or disorders or autoimmune diseases e.g. rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, Hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes type I or II and the disorders associated therewith, respiratory diseases such as asthma or inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, cutaneous manifestations of immunologically-mediated disorders or illnesses, inflammatory and hyperproliferative skin diseases (such as psoriasis, atopic dermatitis, allergic contact dermatitis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis), inflammatory eye diseases, e.g. Sjoegren's syndrome, keratoconjunctivitis or uveitis, inflammatory bowel disease, Crohn's disease or ulcerative colitis. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.1 to about 100 mg/kg body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 2000 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form.

The compounds of formula I may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent. Unit dosage forms for oral administration contain, for example, from about 0.1 mg to about 500 mg of active substance.

Topical administration is e.g. to the skin. A further form of topical administration is to the eye.

The compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by T lymphocytes and/or PKC or GSK-3β, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

2. A compound of formula I, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 and 1.2 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 and 1.2 above comprising a compound of formula I in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula I or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 and 1.2 above.

Compounds of formula I may be administered as the sole active ingredient or together with other drugs in immunomodulating regimens or other anti-inflammatory agents e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders. For example, they may be used in combination with cyclosporines, or ascomycines or their immunosuppressive analogs or derivatives, e.g. cyclosporin A, ISA Tx247, FK-506, ABT-281, ASM 981; an mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578, or a rapalog, e.g. AP23573, AP23464, AP23675, AP23841, TAFA-93, biolimus 7 or biolimus 9 etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; an EDG receptor agonist having

accelerating lymphocyte homing properties, e.g. FTY 720 or an analogue thereof; leflunomide or analogs thereof; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or analogs thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD 11a/CD18, CD7, CD25, CD 27, B7, CD40, CD45, CD58, CD 137, ICOS, CD150 (SLAM), OX40, 4-1BB or their ligands, e.g. CD154; or other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4lg (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y, or other adhesion molecule inhibitors, e.g. mAbs or low molecular weight inhibitors including LFA-1 antagonists, Selectin antagonists and VLA-4 antagonists. Compounds of formula I may also be administered together with an antiproliferative drug, e.g. a chemotherapeutic drug, e.g. as used in cancer treatment, including but not limited to aromatase inhibitors, antiestrogens, topoisomerase I inhibitors, topoisomerase II inhibitors, microtubule active agents, alkylating agents, histone deacetylase inhibitors, farnesyl transferase inhibitors, COX-2 inhibitors, MMP inhibitors, mTOR inhibitors, antineoplastic antimetabolites, platin compounds, compounds decreasing the protein kinase activity and further anti-angiogenic compounds, gonadorelin agonists, anti-androgens, bengamides, bisphosphonates, antiproliferative antibodies and temozolomide, or with an anti-diabetic drug, an insulin secretagogue or insulin secretion enhancer, e.g. a sulphonyl urea, e.g. tolbutamide, chlorpropamide, tolazamide, acetohexamide, 4-chloro-N-[(1-pyrolidinylamino)carbonyl]-benzensulfonamide (glycopyramide), glibenclamide (glyburide), gliclazide, 1-butyl-3-metanilylurea, carbutamide, glibonuride, glipizide, gliquidone, glisoxepid, glybuthiazole, glibuzole, glyhexamide, glymidine, glypinamide, phenbutamide or tolylcyclamide, an oral insulinotropic agent derivative, e.g. a short acting insulin enhancer, e.g. meglitinide, repaglinide, a phenyl acetic acid derivative, e.g.nateglinide, a DPP IV inhibitor, e.g. 1-{2-[(5-cyanopyridin-2-yl)amino]ethylamino}acetyl-(2S)-cyano-pyrrolidine di-hydrochloride, LAF237, GLP-1 or a GLP-1 agonist analog, or an insulin sensitizer e.g. a peroxisome proliferator activated receptor γ agonist (PPARγ), e.g. a glitazone, a non-glitazone type such as a N-(2-benzoylphenyl)-L-tyrosine analogue, e.g. GI-262570, or an oxolidinedione, e.g. JTT501, a dual PPARγ/PPARα agonist, e.g. DRF-554158, NC-2100 or NN-622, a retinoid X receptor agonist or a rexinoid, e.g. 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid, 4-[(3,5,5,8,8-pentamethyl-

5,6,7,8-tetrahydro-2-naphthyl)-2-carbonyl]-benzoic acid, 9-cis retinoic acid or an analog, derivative or a pharmaceutically acceptable salt thereof, in diabetes therapy,

In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I in free form or in pharmaceutically acceptable salt form, and a second drug substance, said second drug substance being an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic drug, e.g. as indicated above.

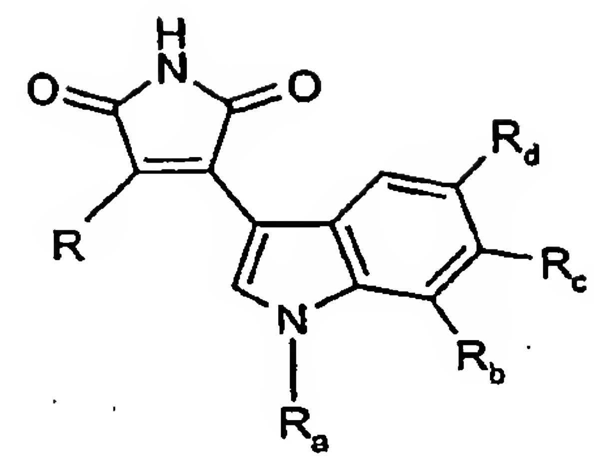
6. A therapeutic combination, e.g. a kit, comprising a) an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I in free form or in pharmaceutically acceptable salt form, and b) at least one second agent selected from an immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative and anti-diabetic drug. Component a) and component b) may be used concomitantly or in sequence. The kit may comprise instructions for its administration.

Where an inhibitor of GSK-3β, PKC or of T-cell activation and proliferation, e.g. a compound of formula I, is administered in conjunction with other immunosuppressive/immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic therapy, e.g. for preventing or treating acute or chronic graft rejection or inflammatory or autoimmune disorders as hereinabove specified, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory, antiproliferative or anti-diabetic compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a cyclosporine, on the specific drug employed, on the condition being treated and so forth.

Compounds of formula I have an interesting pharmacokinetic profile and interesting in vitro and in vivo activities.

CLAIMS

1. A compound of formula I



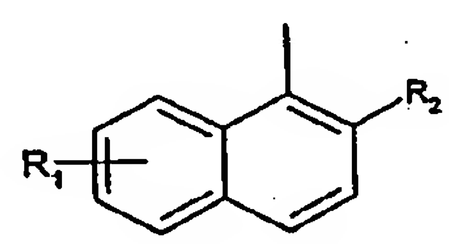
I

wherein

R_a is H; $C_{1\text{-}4}$alkyl; or $C_{1\text{-}4}$alkyl substituted by OH, NH_2, $NHC_{1\text{-}4}$alkyl or N(di-$C_{1\text{-}4}$alkyl)$_2$;

one of R_b, R_c and R_d is halogen; $C_{1\text{-}4}$alkoxy; or $C_{1\text{-}4}$alkyl; and the other two substituents are H; or R_b, R_c and R_d are all H; and

R is a radical of formula (a)



(a)

wherein

R_1 is $-(CH_2)_n-NR_3R_4$ and R_1 is in position 6 or 7,

wherein

each of R_3 and R_4, independently, is H or $C_{1\text{-}4}$alkyl; or R_3 and R_4 form together with the nitrogen atom to which they are bound a heterocyclic residue;

n is 0, 1 or 2; and

R_2 is H; halogen; $C_{1\text{-}4}$alkyl; CF_3; OH; SH; NH_2; $C_{1\text{-}4}$alkoxy; $C_{1\text{-}4}$alkylthio; $NHC_{1\text{-}4}$alkyl; N(di-$C_{1\text{-}4}$alkyl)$_2$ or CN;

or a salt thereof,

a process for its preparation, its use as a pharmaceutical, a pharmaceutical composition containing such a compound or a pharmaceutically acceptable salt thereof, a method of treatment or prevention using such a compound or a pharmaceutically acceptable salt thereof, or a pharmaceutical combination comprising such a compound or a pharmaceutically acceptable salt thereof, substantially as herein defined and/or described.

2. A compound substantially as described in the Examples.

COR
PCT/EP2005/000501